# Summary of State and National
# Biological and Physical Habitat Assessment Methods
# with a Focus on US EPA Region 7 States

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By the
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## OVERVIEW

The primary purpose of this review and summary of State and National biological assessment methods, physical habitat assessment methods, and biological criteria is to allow comparisons to be made between federal and states organizations that are directly or indirectly involved in aquatic resource monitoring and management. In addition to the individual reviews of identified methods, a number of tabular summaries were constructed to facilitate ready comparisons of elements and factors common to all or most reviewed methods. It was envisioned that assessments of current efforts of scientists and environmental management organizations involved in monitoring aquatic resources would provide some useful information concerning common monitoring practices and approaches that are in use including which primary indicator groups and measurement metrics are being used in aquatic assessments in US EPA Region 7. This work summarizes the specific methods used by all regulatory entities (and some non-regulatory groups) within states comprising US EPA region 7 and also includes some state and federal organizations that have well established methods and bioassessment programs. Some of the states included such as Ohio have a long history of bioassessment work and have established biological criteria for use in their state. Specific monitoring and biological assessment documents produced by or for National entities such as US EPA, USGS, and USFS were also reviewed as these agencies have long standing monitoring programs and years of associated aquatic data that could be used in evaluating specific methods.

Much of this review focuses on lotic ecosystems (*e.g.,* streams and rivers) because these are the most common or prominent aquatic ecosystems of these facilities, and documentation of standard bioassessment methods for lentic ecosystems (*e.g.,* ponds, lakes, wetlands) are few. The biological quality of lakes and ponds is often associated with chlorophyll *a* concentrations with persistently high levels (> 10μg/L) being indicative of cultural eutrophication. We have covered some biological methods for lakes and wetlands when these aquatic ecosystems were included in state determined methodologies.

## NATIONAL TRENDS IN BIOLOGICAL ASSESSMENT AND BIOLOGICAL CRITERIA

Monitoring the quality of water resources is best accomplished using an integrated ecological approach. The quality or integrity of an aquatic resource is determined by physical, chemical, and biological factors both instream and in the surrounding watershed. Traditionally water chemistry measurements have been heavily relied upon for monitoring the quality of waters. However, measurements of water column chemical constituents only contain information on the conditions at the time the samples were taken (USEPA 1994). Chemical measurements alone also fail to incorporate the long-term effects of instream, riparian, and watershed-wide habitat degradation. For these reasons the assessment of aquatic biological communities has become a common and effective means to supplement physical and chemical water resource data. The use of macroinvertebrates as biological indicators of water quality and ecological integrity began in the early part of the 20th century (USEPA 1990). Since then the use of biological assessment methods has become a standard tool of the scientific community and regulatory agencies. This is a result of the abundance of research and development that has been devoted to the development of biological monitoring field methods and data analysis methods by various universities, federal agencies, and state agencies. The methods used today are scientifically defensible, applicable in nearly every region of the country, and have become indispensable in monitoring the health of the nation's aquatic ecosystems. Aquatic organisms provide an integrated view of ecological condition because they are often long-lived and sensitive to watershed-wide land use practices and management. For these reasons USEPA has required all states to assess, protect and if necessary restore the biological integrity of their stream resources. In addition EPA requires states to define aquatic life uses, utilize biological monitoring to determine attainment or non-attainment of those designated uses, and define biological criteria in support of those uses.

All 50 states currently have biological assessment programs in place. Fish, macroinvertebrates, and periphyton comprise the major aquatic assemblages utilized by state biological monitoring programs. The most common assemblage used is macroinvertebrates. All 50 states with the exception of Hawaii utilize this group of organisms for biological monitoring. However many states are benefiting from assessing more than one assemblage. USEPA (2002)found that using only one assemblage is only 80-85% effective for identifying attainment or non-attainment of designated uses, and thus recommends using more than one

biological assemblage. As of 2001, 41 states were employing more than one assemblage and of those 20 were using at least three (USEPA 2002).

The major difficulty with biological monitoring is determining from biological data whether or not a sample site is degraded or not, and if it is to what extent. The concept of reference sites or reference conditions have proven very useful in determining the degree of impact a sample site may be experiencing. Reference sites are commonly chosen based on their level of human disturbance. Reference sites are most commonly defined as being the least-disturbed sites within an ecoregion. Reference conditions are determined statistically from an aggregate of data obtained from several least-disturbed sites within a region. For the reference site or condition concept to be effective, control or sample stations must have physical and chemical habitat characteristics similar to the reference site or reference condition. Reference sites or conditions provide a convenient and scientifically defensible yardstick from which to compare the biological condition of control stations. Currently the ecoregion reference condition concept is taking precedence over the use of site-specific reference conditions. In 1995 only 15 states had integrated ecoregional reference conditions into their biological monitoring programs, but by 2001, 39 states were utilizing this concept (USEPA 2002).

State and Federal biological monitoring programs commonly employ two basic methods of data analysis; these are the multimetric and multivariate approaches. Multimetric and multivariate analysis approaches are used to reduce large amounts of environmental and biological data into numeric values associated with biological condition. Biological metrics are indices that are expected to increase or decrease in value in response to increases or decreases in environmental perturbation or in response to specific environmental stressors. Commonly several metrics are combined to form an additive multimetric index of biological or ecological integrity. Multivariate approaches are statistical techniques, which determine the relationships of several variables simultaneously. Of 54 state and tribal entities surveyed by (USEPA 2002), 41 employed the multimetric approach. Biological multimetric indices and multivariate approaches are useless without integrating habitat condition data. Most states utilize a visual based habitat assessment method to support biological data. Methods such as Ohio EPA's Qualitative Habitat Evaluation Index (QHEI) and USEPA's Rapid Bioassessment Protocol (RBP) are commonly employed by state agencies because they have been proven useful in nearly all regions of the country and are scientifically sound. In addition to the visual based assessment most entities also use quantitative measurements that characterize the stream channel under study and provide a measurable basis for detecting changes that may be associated with biological condition change.

Finally, the Clean Water Act (Federal Water Pollution Control Act of 1972, Clean Water Act of 1977, and the Water Quality Act of 1987) requires States to develop biological criteria; sections 303 and 304 provide the basis for development of biological criteria. Most states have developed and implemented narrative biological criteria, often in an antidegradation statement in their water quality standards. Some have developed biological criteria with specific reference to biological conditions and most have some form of specific criteria under development. Few states have developed numerical criteria based on a multimetric or multivariate biological assessment approach. Of the state entities presented in this report only Ohio has developed and implemented numeric biological criteria.

# SUMMARY OF BIOLOGICAL ASSESSMENT METHODS USED BY STATES WITHIN USEPA REGION 7

# I. STATE OF IOWA

Protocols are presented in a variety of state documents (Iowa Department of Natural Resources 1994; Iowa Department of Natural Resources 1999).

## SAMPLING REACH CONSIDERATIONS

Sample reaches for streams vary from 150 meters to 500 meters depending on stream size and habitat type frequency. Guidelines suggest that the reach should include 3 distinct pool and riffle habitats. In streams where pool and riffle sequences are unavailable then the reach should include three well-defined channel bends.

## MACROINVERTEBRATE COMMUNITY ASSESSMENT

Iowa Department of Natural Resources utilizes both semi-quantitative and qualitative sampling of macroinvertebrates to assess the biological integrity of streams. A macroinvertebrate index of biological integrity (MIBI) is used for data analysis and interpretation, however metrics for the MIBI were not provided. Three replicate samples are taken at each site and are analyzed as separate samples.

FIELD METHODS AND INDEX PERIOD

Semi-Quantitative:

> Modified-Hess samplers or Surber samplers are used in streams and rivers with riffles and runs less than 1.5 feet deep. The samples are not composited.

> Modified Hester-Dendy samplers are used in streams where riffle habitat is absent. Three modified artificial substrates consisting of eight 1/8" × 3" × 3" wood plates and twelve 1" square wooden spacers are placed at a sampling site. The total area of artificial substrate available for colonization at each sample site is 145.6 square inches. The samplers are deployed in water 1 to 2 feet deep having a velocity of 0.5 to 1.5 feet per second. The colonization period is a minimum of 4 weeks and a maximum of 6 weeks. The samples are not composited.

Multi-habitat Sampling - Qualitative

> Qualitative sampling is performed to supplement the semi-quantitative sampling by provide better representation of taxa throughout the sample reach. This is performed the same day artificial substrates are retrieved. The mesh size of sieves, wash-buckets, and kick nets should be 500 to 600 microns. Sampling is performed from all available natural habitats concentrating on those that provide the highest amount of diversity, *e.g.*, riffles and woody debris snags. The qualitative samples are composited in the field. Stream sampling for macroinvertebrates is conducted annually between July 15 and October 15.

Field Preservation

> Samples are preserved in the field with a 10% formalin solution buffered with 3 grams of borax to each liter of formalin solution.

LABORATORY METHODS

Laboratory Preservation

> Prior to identification and enumeration of the samples the organisms are transferred from the 10% buffered formalin solution to 85% ethanol solution.

Subsampling

> Random 100-organism subsamples.  Each of the triplicate Modified Hess samples are subsampled individually.  The qualitative composite sample is subsampled.

Level of Taxonomic Identification

> Lowest practical level depending on the abundance of difficult to identify organisms such as chironomids and oligochaetes, time constraints, and availability of taxonomic keys.  Dipteran midge larvae (Chironomidae) are not currently identified beyond family.

## DATA ANALYSIS AND INTERPRETATION

Iowa is in the process of finalizing the development and adoption of a multimetric index for use in assessing macroinvertebrate community health for Iowa's streams and rivers.  They evaluated about 39 potential metrics and produced a final index consisting of 12 (Table 1).

Table 1. Summary of Iowa Department of Natural Resources' macroinvertebrate metrics and some of their characteristics.  Multi-habitat (MH) and single habitat (SH) derived metrics are indicated in the table. All percents are based on number of organisms not number of taxa.

| Data Metric | Metric variability (sampling error) | Impacted Site Discriminatory Power |
|---|---|---|
| MH – taxa richness | low | moderate |
| MH – EPT richness | low | high |
| MH – sensitive taxa | moderate | high |
| SH – taxa richness | low | moderate |
| SH – EPT richness | low | high |
| Percent of 3 dominant taxa | low | high |
| Biotic index | low | high |
| Percent EPT | low | low |
| Percent Chironomidae | high | high |
| Percent Ephemeroptera | moderate | moderate |
| Percent scrapers | moderate | moderate |
| Percent dominant functional feeding group | low | moderate |

A metric score procedure was established so that each metric scoring range was from 0 –10 and the macroinvertebrate index (BM-IBI) has a possible range from 0 to 100.

## FISH COMMUNITY ASSESSMENT

### FIELD METHODS AND INDEX PERIOD

Single pass electrofishing.  Second pass may be performed if results of first pass are unsatisfactory.  Fish are captured using ¼" mesh nets.  Time sampled in seconds and length and average width of the stream reach sampled are recorded to provide CPUE data.  Fish are preserved in 10% formalin solution.  Fish sampling is most often accomplished from spring through fall (*e.g.,* October), and one sample is collected per site.

### LABORATORY METHODS

Fish are identified to species.

### DATA ANALYSIS AND INTERPRETATION

Elements analyzed include: species composition, number of species, relative abundance of species, catch per unit effort, proportion of fish sampled with external abnormalities such as parasites, lesions, eroded fins, or deformities.

**PHYSICAL HABITAT ASSESSMENT**

The habitat assessment is performed in conjunction with the macroinvertebrate and fish sampling. Two forms are used. One is used for assessment of the habitat in which the semi-quantitative macroinvertebrate sampling devices were deployed and the other for the assessment of habitats sampled during the course of the qualitative multi-habitat macroinvertebrate samples. Together the assessment includes subjective estimates of periphyton growth, dominant type of periphyton, amount of sedimentation and a ranking of the five most abundant types of benthic substrates in the sample reach.

**REFERENCE SITE SELECTION**

The documentation of a reference conditions and site selection process is ongoing.

**BIOLOGICAL CRITERIA**

Under Development.

## II. STATE OF KANSAS

**SAMPLING REACH CONSIDERATIONS**

Kansas Department of Wildlife and Parks (KDWP) conducts fish and macroinvertebrate sampling as part of USEPA Regional Environmental Monitoring and Assessment Program (REMAP) projects and utilizes the protocols designed for that program, including sample reach definitions and considerations (USEPA 1998). Kansas Department of Health and Environment (KDHE) conducts macroinvertebrate sampling in support of their water quality programs. The KDHE protocols used are presented below.

**MACROINVERTEBRATE COMMUNITY ASSESSMENT**

FIELD METHODS AND INDEX PERIOD

KDHE field collections are made by two people sampling over a period of 30 minutes for a total of one person/hour sample effort at a site. All available macrohabitats are sampled using D-frame invertebrate nets with 500-micron mesh nets. Kick sampling; sweep sampling, and sieving fine sediments through the net are viable collection techniques used with the D-frame net. Directly picking invertebrates from large substrates is also used. The total sample should contain 200 or more total organisms, with no more than 50 organisms being obtained from any single microhabitat. Each person collects and stores their samples in their own individual sample container. Samples are collected in the spring of the first year, the summer of the next, and the fall of the next, the cycle is repeated every three years. The typical index period is May through September but can extend into October.

Field Preservation

  70-80% ethyl alcohol (ETOH).

LABORATORY METHODS

Laboratory Preservation

  70-80% ETOH with 5% glycerin. Samples are retained for two years.

Subsampling

  No subsampling in the laboratory. The samples obtained individually by the two field collectors are considered as two subsamples.

Level of Taxonomic Identification

  Lowest possible level.

DATA ANALYSIS AND INTERPRETATION

The results of the two 30 minute sample efforts are compared as an indication of sampling precision. KDHE uses number of taxa; EPT ratio; KBI using tolerance values for nutrient and oxygen-demanding substances (see Huggins and Moffett 1988); and the MBI as metrics for quantifying macroinvertebrate communities. The MBI is a family/order level biotic index based on the Hilsenhoff Biotic Index (Hilsenhoff 1987). Percent mussel taxa loss is also used and typically all metrics are given equal weight in 305(b) evaluations.

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**FISH COMMUNITY ASSESSMENT**

No related material for KDHE.  Kansas Department of Wildlife and Parks as part of the EPA Region 7 REMAP program use USEPA REMAP protocols for the evaluation of stream fish communities.

**PHYSICAL HABITAT ASSESSMENT**

KDHE utilizes a simple subjective habitat development index (HDI).  Macrohabitat types are given a score of 3 if present or a score of 0 if not present.  Average depths of the macrohabitats are scored from 0 to 2 according to a list of categories on the HDI form.  Scores are generated for categories of riffle substrate size and level of embeddedness.  The type and quantity of organic debris are scored on a scale of 0 to 3. Filamentous algal masses are scored 0 for absence or 1 for presence, with limited epiphytic biological growths scored as 0 as they provide little physical shelter.  Macrophytes are scored from 0 to 2 depending on presence, absence, and quantity.  Bank vegetation is scored from 0 to 2 depending presence, absence, and the quantity adjacent to each sampled macrohabitat.  The total score on the form is tabulated to derive a sample score.

Kansas Department of Wildlife and Parks uses an HDI that derives a total stream quality score based on subjective ratings of several variables within each of four basic habitat quality components.  The first component is physical habitat including ratings for flow, substrate types, substrate quality, instream cover, macrohabitat presence/absence, and bank erosion.  The second component is riparian/floodplain quality including estimates of riparian vegetation, canopy cover, and adjacent land use categories.  A biological component is included which requires data on fish, macroinvertebrates, mussels, and amphibians.  The final component is pollution as indicated by Secchi depth, evidence of pollution other than silt, and whether chemical variables measured in the field are considered limiting or non-limiting to the aquatic community.

**REFERENCE SITE SELECTION**

The Kansas Department of Health and Environment KDHE (1995) defines a reference site as one that "represents an unusually pristine location, suitable for use as a long-term ecoregional reference location." Their intended use is to "identify the variation in community structure and species abundance associated with relatively unperturbed streams in a given land use setting, geological or geographical area, or ecoregion."  Reference site data in Kansas are compared to invertebrate community data from sample sites to determine if the community is degraded and affected by water quality problems.

**BIOLOGICAL CRITERIA**

Under Development.

# III. STATE OF MISSOURI

Methodology is detailed in a number of state documents (Missouri Department of Natural Resources - Division of Environmental Quality 1994; Missouri Department of Natural Resources 1998; Missouri Department of Natural Resources 1998; Missouri Department of Natural Resources 1998).

## SAMPLING REACH CONSIDERATIONS

Macroinvertebrate bioassessment methods are intended for use in streams with an average depth of less than 1.5 meters.  Only habitats that are commonly found in the study reaches are sampled, uncommon habitats are not sampled.  Sampling reaches are defined as twenty times the average stream width.  Two comparable reaches within three stream miles are sampled for community characterization.

## MACROINVERTEBRATE COMMUNITY ASSESSMENT

### FIELD METHODS AND INDEX PERIOD

Missouri utilizes methods obtained from EPA RPB and the North Carolina Division of Environmental Quality.  These methods provide a multi-habitat semi-quantitative approach.  The following sample types are taken at a sample site.  Macroinvertebrates are collected between September 15 and October 15, which coincides with stable baseflow conditions.

Flowing water – coarse substrates
> Kick samples using D-frame net with 500-micron mesh
> Approximately one square meter each sample
> Six-sample composite
> Variety of microhabitats sampled

Non-flowing water
> Kick samples using D-frame net with 500-micron mesh
> Approximately one square meter each sample
> Six-sample composite
> Variety of microhabitats sampled

Vegetation
> Kick samples using D-frame net with 500-micron mesh
> Approximately one square meter each sample
> Six-sample composite
> Variety of microhabitats sampled

Flowing water – fine substrates
> Kick samples using D-frame net with 500-micron mesh
> Approximately one square meter each sample
> Twelve-sample composite
> Variety of microhabitats sampled

Leaf packs
> Six handfuls are collected and elutriated through a brine shrimp net
> Variety of flow conditions are sampled
> No mention of compositing samples in document

Snags
> Twelve-sample composite
> 400 × 600 square centimeters each sample
> Organisms are brushed off of snag material into a 500-micron mesh bag
> Variety of flow conditions and log deterioration states are sampled

Root mats and undercut banks
> Kick samples using D-frame net with 500-micron mesh
> Approximately one-meter strip of shoreline for each sample

Six-sample composite
Variety of microhabitats sampled

Field Preservation

10% Formalin solution

## LABORATORY METHODS

Level of Taxonomic Identification

Lowest possible level.

Laboratory Preservation

80% ETOH is used during subsampling and identification.
70% ETOH is used for permanent storage of reference specimens.

Subsampling

600-organism subsample for coarse substrate samples taken in flowing water..
300-organism subsample for all other habitat samples.

Organisms are subsampled from composited samples using a grid system in large plastic trays.
Grids are chosen using a random number generator.

## DATA ANALYSIS AND INTERPRETATION

Large/rare species are only used in the calculation of Taxa Richness and EPT Taxa Index values;
they are omitted from the calculation of all other metrics.

Stream Condition Index (SCI) is a multimetric approach used to quantify the biological condition
at sample sites. Usually only the four primary metrics listed are used (see Table 2), only in special
circumstances are secondary metrics used. A minimum of six reference site samples is used to
form a distribution of metric scores. The range of these score distributions is divided into four
quartiles, with the upper bound of the lower quartile defining an approximation of reference
condition. Metrics calculated from study sites that score above the reference condition assigned a
value of 5, the range of values below the reference condition are divided in half and assigned
values of 3 and 1. The sample site metric scores are totaled for the final SCI site score.
Interpretation of the final score is currently under development.

Quality control is achieved by collecting duplicate samples at 10% of the sample sites and they are
analyzed for similarity with the regular samples.

Table 2. Macroinvertebrate metrics used in the Missouri Department of Natural Resources Stream
Condition Index.

| Primary Metrics | Response to Increasing Water Quality |
|---|---|
| Taxa Richness | Increase |
| EPT Taxa Index | Increase |
| Biotic Index | Decrease |
| Shannon's Diversity Index | Increase |
| **Secondary Metrics** | **Response to Increasing Water Quality** |

| | |
|---|---|
| Quantitative Similarity Index for Taxa (QSI-T) | Increase |
| Pinkham and Pearson Similarity Index (PPSI) | Compared to Reference Site Data |
| Percent Dominant Taxa | Decrease |
| Dominants in Common | Compared to Reference Site Data |
| Percent Scrapers | Decrease |
| Quantitative Similarity Index for Functional Feeding Groups (QSI-FFG) | Compared to Reference Site Data |

**FISH COMMUNITY ASSESSMENT**

No related material.

**PHYSICAL HABITAT ASSESSMENT**

Missouri's Department of Natural Resources habitat assessment is a modification of EPA RPBs. Three main categories of habitat parameters are measured or estimated. Primary parameters characterize microhabitat scale features and are scored on a scale of 0-19. Secondary parameters characterize macrohabitat scale features and are scored on a scale of 0-15. Tertiary parameters characterize riparian and bank features and are scored on a scale of 0-11. This scoring scheme weighs primary and secondary parameters above tertiary parameters. The final score of a sample site is compared to nearby or regional reference stream data and interpreted as comparable to reference conditions or three lower levels of supporting conditions. For low gradient streams, two primary parameters and one secondary parameter are substituted.

Table 3. List of Primary, Secondary, and Tertiary parameters for use in high gradient riffle/run prevalent streams and substitution parameters for use in glide/pool prevalent low gradient streams utilized by the Missouri Department of Natural Resources.

| Riffle/Run Prevalent High Gradient | Glide/Pool Prevalent Low Gradient Parameter Substitutions |
|---|---|
| **Primary Parameters** | **Primary Parameters** |
| Bottom Substrate/Instream Cover | |
| Embeddedness | Pool Substrate Characterization |
| Stream Flow or Velocity/Depth Regime | Pool Variability |
| Canopy cover | |
| **Secondary Parameters** | **Secondary Parameters** |
| Island and Point Bar Growth | |
| Bottom Scouring and Deposition | |
| Riffle to Riffle/Width Ratio | Channel Sinuosity |
| Lower Bank Channel Capacity | |
| **Tertiary Parameters** | |
| Upper Bank Stability | |
| Bank Vegetative Stability/Grazing Pressure | |
| Streamside Cover | |
| Riparian Vegetative Zone Width | |

Table 4. Missouri Department of Natural Resources habitat assessment score interpretation. The score is interpreted by its percent similarity to local or regional reference conditions.

| Percent Similarity to Reference Condition | Interpretation |
|---|---|
| ≥ 90% | Comparable to Reference |
| 75-89% | Supporting |
| 60-74% | Partially Supporting |

**REFERENCE SITE SELECTION**

Definition

Reference sites are defined as having as little human disturbance as possible.

Identification

Potential reference sites are selected if the habitat quality of the potential site is comparable to established reference conditions. The total score from the physical habitat assessment must be greater than or equal to 90% similar to established reference conditions to be considered a reference site.

**BIOLOGICAL CRITERIA**

Narrative

The biological integrity of waters, as measured by lists or numeric diversity indices of benthic invertebrates, fish, algae, or other appropriate indicators shall not be significantly different from reference waters. Waters shall be compared with reference waters of similar size within an ecoregion.

Numeric

None

# IV. STATE OF NEBRASKA

Methodologies are detailed in a number of state documents (Nebraska Department of Environmental Quality 1992; 1993; Nebraska Department of Environmental Quality 1997; Nebraska Department of Environmental Quality 1999).

## SAMPLING REACH CONSIDERATIONS

Sampling reach should contain all available habitats needed to obtain a representative sample of fish and macroinvertebrates.

## MACROINVERTEBRATE COMMUNITY ASSESSMENT

### FIELD METHODS AND INDEX PERIOD

Both qualitative and quantitative methods are used dependent upon monitoring objectives. While no specific collection period was noted, inspection of their data suggests that nearly all macroinvertebrate samples were collected from May through September. The number of macroinvertebrate samples collected during routine monitoring efforts appears to be limited to a single sample.

Qualitative Sampling

> Sampling equipment includes No. 30 mesh screen or D-frame net. Sampling is conducted for 15 to 30 minutes in all available habitat types. Hand picking of larger substrates such as rocks and woody debris is also recommended.

Semi-Quantitative Sampling

> Nebraska Department of Environmental Quality (NDEQ) uses EPA Rapid Bioassessment Protocols (Barbour, Gerritsen et al. 1999).

> Sampling equipment includes No. 30 mesh screen or D-frame net. All available habitats sampled for a known length or area.

> Ponar grab sampler, Surber sampler, Hester-Dendy multiple plate artificial substrate, Peterson grab sampler. D-frame kick nets are used to collect from a known area.

Field Preservation

> 10% Formalin or 70 ETOH

### LABORATORY METHODS

Laboratory preservation

> Samples are stained with rose Bengal (100g/L) in either 10% formalin or 70% ETOH. Samples are retained for three years after collection.

> No. 30 mesh screen sieve for washing samples.

> Sugar may be added to 70% ETOH or 10% formalin solution to aid in floating organisms free of debris.

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Chironomidae, Naididae, and Tubificidae are mounted with CMC-10 mounting media on a glass slide with a cover slip.

Subsampling

Field samples are washed in No. 30 (500 micron) mesh screens. Subsampling is only conducted in cases where excessive amounts of specimens are contained in the sample.

Subsamples are obtained by mixing a field sample in a container rapidly, and then the sample is poured into a dish with an evenly marked grid. From this grid a percentage of the sample is removed at the discretion of the analyst. The unused portion is then examined for new and unusual specimens not included in the subsample. Each subsample should contain no fewer than 100 organisms.

In samples dominated by very large number of individuals from a single taxonomic group, all organisms are separated and counted. 70 organisms are then removed from the total sample and the ratio of the various taxa is then calculated to determine the approximate number of each taxon in the total sample.

Biomass

Biomass per unit area is calculated for samples taken with semi-quantitative sampling equipment. Dry weight and ash-free dry weight are measured.

Level of Taxonomic Identification

Lowest possible level.


DATA ANALYSIS AND INTERPRETATION

The following indices are calculated:
Shannon-Weaver diversity index.
Dry weight and ash-free dry weight are reported as grams per square meter.
Hilsenhoff Biotic Index
Jaccard Coefficient of community Similarity
Invertebrate Community Index (ICI) (Plafkin, Barbour et al. 1989; Barbour, Gerritsen et al. 1999).


Table 5. Invertebrate Community Index metrics used by Nebraska Department of Environmental Quality.

| | |
|---|---|
| Taxa Richness | Shredder/Total Number Individuals Ratio |
| EPT Taxa Richness | EPT/Total Taxa Index |
| Chironomid Taxa Richness | EPT/Chironomidae Abundance |
| Hilsenhoff Biotic Index | Community similarity Indices |
| EPT/Chironomid + EPT Ratio | Community Loss Indices |
| Percent Dominant Taxa | Index of Similarity Between Two Samples |
| Scraper to collector + Filterer Ratio | Pinkham and Person Community Index |
| Jaccard Index | Other Similarity Indices |
| Scraper/Filterer + Scraper Ratio | Presence/Absence of Specific Indicator Organisms |

Data Quality Control

Complete samples are re-identified by a different analyst. The difference in the numbers of individual taxa and the relative abundance of the taxa must fall within 20 percent of the original

identification effort.  All debris present during the first identification will be retained for the second identification to ensure replicability.

**FISH COMMUNITY ASSESSMENT**

<u>FIELD METHODS AND INDEX PERIOD</u>

Semi-quantitative sampling methods are employed where all habitat types are sampled in a reach of sufficient length and for a time adequate to obtain a representative sample from the reach.  Collection of fish samples for bioassessment purposes is most desirable between late May and early September.

Seining

No guidelines provided for seine size, mesh size, or number of passes.  Electric seines are cited as being more efficient than seines and backpack electrofishing units.

Electrofishing

Boat electrofishing rigs, boat mounted (tote barge), and backpack units using either DC or AC current.  Document favors DC.  Sampling in a downstream direction is recommended for electrofishing from a boat in a stream or river.

Passive sampling equipment

Gill nets, hoop nets, and fyke nets.  Gill nets are recommended for shallow areas with no current.  Hoop nets are recommended for sampling rivers with strong current.  Fyke nets are recommended for sampling shallow areas in ponds and lakes.

Block nets are recommended for isolating the stream fish communities.

Field Preservation

10% formalin is used for preservation in the field.

Field Fish Processing

Fish that are readily identifiable to species are identified in the field and released.  Total lengths and weights are recorded for game species.  Fish that are not readily identifiable in the field are identified in the laboratory.

<u>LABORATORY METHODS</u>

Preservation

70% ETOH is used for laboratory preservation.

Level of taxonomic identification

Species level.

<u>DATA ANALYSIS AND INTERPRETATION</u>

Catch per unit time and stream length sampled are calculated.

The Index of Biological Integrity (IBI) as defined and refined by Karr *et al.* (1986) is used by NDEQ.  The IBI is scored based on comparisons between the individual metric values at control

sites to those expected from reference conditions given a stream of similar size in a similar geographic region. A value of (5) is given if the metric approaches that expected at a reference site, (3) if it deviates somewhat, and (1) if it deviates strongly from values expected at a reference site. The following values may be calculated for analysis of fish community data.

Table 6. Fish Index of Biological Integrity metrics used by the Nebraska Department of Environmental Quality.

| Community Diversity Indices | |
|---|---|
| Shannon-Weaver index | Simpson Values for Nonrandom Samples |
| Margalef Diversity | Shannon Diversity and Evenness |
| Menhinick Diversity | Brillouin Diversity and Evenness |
| Simpson Dominance | Heip Evenness |
| Simpson Diversity and Evenness | Sheldon Evenness |
| Inverse Simpson Dominance and Evenness | |
| **Community Similarity Indices** | |
| Jaccard Coefficient | Sorensen Coefficient |
| Percent Similarity | Morisita Index |
| Dissimilarity Index | Horn Index |

Population Estimation

> Peterson Method – This is a mark-recapture method, where the fish captured at a sampling event are marked in some way, usually a fin clip, then the population is sampled again and marked individuals are counted as recaptures. A population estimate can then be calculated from the proportion of marked individuals to unmarked individuals.

> Schnabel Method – This is mark-recapture method using the multiple-census method where fish are marked and recaptured several times within the same population. This method is best used on large bodies of water for increasing the confidence interval of the samples.

> Leslie Method – This is a depletion sampling method where the population, *e.g.*, in a small pond or blocked section of stream are sampled to point where the catch per unit effort (CPUE) is diminished. Linear regression is then used on the subsequent sampling passes to obtain a population estimate.

**PHYSICAL HABITAT ASSESSMENT**

Physical habitat characteristics are estimated subjectively and measured directly depending on the variable. The observations include flow, channel alteration, bank stability, habitat degradation rating, substrate composition, stream width, stream depth, pool description, land use, grazing damage, riffle and run occurrence, stream cover, stream stage, water clarity, runoff influence, aquatic vegetation composition, vegetation stability, and stream order.

Simple subjective ranked assessment of stream quality and a simple inventory of the habitat in support of fish.

NDEQ also conducts a "Biological Network Reconnaissance and Habitat Survey" with several subjective ratings of 1) watershed conditions such as topography, land use, point sources, and land treatments, 2) riparian conditions including general and stream bank, 3) general stream conditions including flow and channel conditions, water quality, and instream habitat measurements taken at three transects.

A "Biological Network Aquatic Vegetation Survey" is conducted where species of emergent and submergent vegetation present in the sample reach are recorded.

Additional measurements or subjective observations are vegetative riparian zone width, bank stability, degradation rating, instream substrate size, stream width, stream depth, stream stage, vegetation occurrence, grazing damage, riffle/run occurrence, pool description, sand bars, land use, and stream cover.

## REFERENCE SITE SELECTION

Definition

Reference sites as defined by NDEQ should meet the following conditions: 1) least impacted site typical of ecoregion and stream type conditions, 2) unimpacted by point sources, 3) unimpacted by nonpoint source activities, and 4) habitat conditions represent the best that is presently achievable.

Identification

Reference sites are determined in the field using a standardized evaluation form consisting of 7 subjective threshold yes/no questions, any of which answered as "no" will disqualify the site. It also contains 7 subjective ratings of various instream, watershed, and riparian factors, which are scored on a scale of 1-7. An index value is determined by adding all rating scores and a site is rejected if the index score is below a specific threshold value.

## BIOLOGICAL CRITERIA

Narrative

Any human activity that would significantly impact or displace an identified "key species" shall not be allowed.

DEFINITIONS

Key species are identified endangered, threatened, sensitive, or recreationally important aquatic species. Key species are designated by stream segment.

Numeric

None

# SUMMARY OF NATIONAL BIOLOGICAL ASSESSMENT METHODS

# I. UNITED STATES ENVIRONMENTAL PROTECTION AGENCY: ENVIRONMENTAL MONITORING AND ASSESSMENT PROGRAM (EMAP) METHODS.

The EMAP methodology (USEPA 1998) was designed to provide a protocol that may be used to conduct a one-site per day assessment with a field crew of four people. These methods were used in USEPA's EMAP from 1993 to 1998 and are used in modified versions for current state, regional, and national projects.

## SAMPLING REACH CONSIDERATIONS

Sample reaches are defined as 40 times the average wetted width or 150 m, whichever is greater. Eleven cross-sectional transects divide the reach into ten equal portions and the transect lines provide points of measurement for assessment activities. Sample reaches are not moved to avoid man-made stream changes such as bridges, culverts, rip-rap, or channelized sections.

## MACROINVERTEBRATE COMMUNITY ASSESSMENT

### FIELD METHODS AND INDEX PERIOD

Sample points are located at the nine interior transect lines starting at the downstream-most interior transect line. It is determined randomly whether the first sample will be taken from the left , right, or center of the transect. Left, center, and right sample points correspond to 25%, 50%, and 75% distance across the wetted width. Once this is determined subsequent samples are taken at points following the left, center, and right sequence moving up the reach until the last of the nine transects is completed. Riffle/run and pool/glide samples are composited respectively for the entire reach and stored in a single container.

Each sample is a 20 second kick sample from a quadrant one net width wide and two net widths long giving a sampled area of approximately 0.5 m$^2$. A D-frame kick net with a net width of 0.5 m and having 600-micron mesh is used for sampling, the width of this net is not the standard 0.3-meter width that is common. A U.S. standard number 30 sieve is used for cleaning the field composited samples.

No guidelines presented for index period.

Field Preservation

     70% ETOH.

### LABORATORY METHODS

No guidelines presented.

### DATA ANALYSIS AND INTERPRETATION

No guidelines presented.

## FISH COMMUNITY ASSESSMENT

### FIELD METHODS

The sample reaches are isolated with block nets. The reach is electrofished first using a backpack electrofishing unit. Seining is conducted after electrofishing. In streams having high habitat complexity and/or very large wadeable streams, intertransect sampling efforts are proportional to the mean intertransect

zone width.  The total amount of effort expended on fish sampling should be no less than 45 minutes in small streams and no more than 3 hours in the largest streams.

Field Preservation

10% Formalin solution.

<u>LABORATORY METHODS</u>

No guidelines presented.

<u>DATA ANALYSIS AND INTERPRETATION</u>

No guidelines presented.

**PERIPHYTON COMMUNITY ASSESSMENT**

<u>FIELD METHODS</u>

Periphyton include algae, fungi, bacteria, and protozoa and are employed as indicators of ecological condition in the EMAP program.  Periphyton samples are collected at the nine interior transects of the sampling reach.  Two basic types of samples are collected from erosional and depositional habitats.  Erosional habitat samples are collected from a large piece of hard substrate such as a rock or piece of wood.  An area delimiter is used for periphyton sample collection. An area delimiter is a ring with an inside area of 12 cm$^2$.  This device is placed on the substrate and the inside area is scrubbed with a toothbrush to dislodge the periphyton.  The dislodged material is then funneled into a 500 ml sample bottle.  In depositional habitats with fine substrates such as sand or silt, the area delimiter confines an area, then the top 1 cm of sediment  is withdrawn from the delimiter using a 60 ml syringe.   The contents of the syringe are then deposited into a 500 ml sample bottle.

<u>LABORATORY METHODS</u>

Both erosional habitat and depositional habitat samples are composited into one sample.  Four further sample types are then created from each of the composited habitat type samples.  1) ID/enumeration samples which are used to determine taxonomic composition and the relative abundances of each taxa, 2) a sample for analysis of chlorophyll a, 3) a biomass sample analyzed as ash-free dry weight, and 4) an acid/alkaline phosphatase activity (APA) sample.

Laboratory preservation

Periphyton samples used for ID/enumeration analyses are preserved with 10% formalin solution.

**PHYSICAL HABITAT ASSESSMENT**

The following seven general habitat attributes are measured or estimated.   Channel dimensions, channel gradient, channel substrate size and type, habitat complexity and cover, riparian vegetation cover and structure, anthropogenic alterations, and channel-riparian interaction.

The EMAP procedure for measuring stream channel characteristics is data intensive and thorough.  100 to 150 thalweg measurements are made at evenly spaced intervals along the stream reach.  Wetted width is measured at each of the 11 transects and at a point in between each transect for a total of 21 measurements.  Slope and backsite bearing are measured between each pair of transects.

Large woody debris such as tree branches, root wads, and logs are tallied in according to defined length and diameter classes at each of the 10 intertransect zones.  Separate woody debris tallies are made in these zones for woody debris found below and above the bankfull height.

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Channel and riparian characterization measurements are made at each of the 11 transects. Measurements include bank height, bank undercut distance, bank angle, bar width, riparian canopy density, substrate size class, substrate embeddedness, areal cover class and type, mid-layer and ground cover, areal cover class of fish concealment features, aquatic macrophytes, filamentous algae, human disturbances, and discharge.

Following the completion of all field activities at a sample site, including the habitat assessment generalized above, a rapid habitat and visual stream assessment utilizing EPA's RPB protocol is conducted to provide further documentary support to the overall assessment.


**REFERENCE SITE SELECTION**

EMAP methods are designed to provide data to help define reference conditions, but no specific guidance is given as to how these definitions should be developed.

## II.  UNITED STATES ENVIRONMENTAL PROTECTION AGENCY:
## RAPID BIOASSESSMENT PROTOCOLS (RBPs)

EPA Rapid Bioassessment Protocols (RBPs) were designed as an inexpensive tool for determining whether streams are supporting or not supporting designated aquatic life use (Barbour, Gerritsen et al. 1999). However, they are also useful for characterizing stream impairment and determining the causes of impairment.  They may also provide data in support of control and mitigation efforts, use attainability, cumulative impact studies, and characterizing regional reference conditions.

## SAMPLING REACH CONSIDERATIONS

A 100-meter reach length is recommended for both single and multihabitat sampling.  Alternatively the EMAP definition of a sample reach, *i.e.,* 40 times the average stream width may be used.  It is also recommended that sample reaches should be at least 100 meters upstream of any anthropogenic disturbances such as bridges, low water dams, or rip-rap piles.  The biological sampling methods employed in a given stream should be based on the habitat availability in reference streams in the same region (ecoregion), thus ensuring that sampling methods are uniform.

## MACROINVERTEBRATE COMMUNITY ASSESSMENT

USEPA's macroinvertebrate rapid bioassessment protocols are the most widely used set of protocols utilized by state agencies responsible for monitoring aquatic resources.  The various methods presented in the RBP's are suitable for the effective sampling of streams having a wide range of habitat structure and complexity, gradients, and flow regimes.

FIELD METHODS AND INDEX PERIOD

Single Habitat Approach

> Riffle and run habitats, if available, should be the target sampling areas of the single habitat approach.  A 1 square meter kick net with 500 micron mesh attached to two poles, analogous to a small fish seine, is used.  One square meter of substrate is disturbed upstream of the kick net.  Alternatively, if a D-frame invertebrate net is used several kicks are composited for each sample location.  No set number of samples is recommended, however it is recommended that at least 2 to 3 samples are taken at various points in a riffle or run based on variations in velocity/depth characteristics or from a series of riffles or runs.

Multiple Habitat Approach

> The multihabitat approach should be used in streams with highly variable habitat characteristics especially when comparing streams in regions with highly variable streams in terms of habitat structure and flow regimes, and gradient.  A D-frame net with 500-micron mesh is used to sample all available habitat types.  20 kick samples or jab samples are taken from all major habitat types.  The 20 samples should be distributed proportional to the percentage of available major habitats.  The total area in square meters sampled should be approximately 3.1.  This approach should target the most productive major habitat types from which the highest diversity of macroinvertebrates is likely to be found.  These habitat types include cobble and gravel, snags, vegetated banks, submerged macrophytes.  In many low gradient streams it may be necessary to sample soft/fine sediments.  The samples are composited into a single sample in the field.

Index Period

> No guidelines presented.

Field Preservation

95% ETOH diluted by water in sample.

<u>LABORATORY METHODS</u>

Level of Taxonomic Identification

Lowest practical level.

Laboratory Preservation

70% ETOH.

Subsampling

Subsamples consist of at least 200 organisms.  A pan marked with a grid system is subsampled using random numbers until the desired number of organisms is achieved.

<u>DATA ANALYSIS AND INTERPRETATION</u>

Table 7. EPA's RBP best candidate benthic macroinvertebrate metrics and predicted responses to increasing stream perturbation.

| Metric Category | Metric | Predicted response to increasing perturbation |
|---|---|---|
| **Richness Measures** | | |
| | Total no. of Taxa | Decrease |
| | Number of  EPT Taxa | Decrease |
| | Number of Ephemeroptera Taxa | Decrease |
| | Number of Plecoptera Taxa | Decrease |
| | Number of Trichoptera Taxa | Decrease |
| **Composition Measures** | | |
| | % EPT Taxa | Decrease |
| | % Ephemeroptera | Decrease |
| **Tolerance/Intolerance Measures** | | |
| | Number of Intolerant Taxa | Decrease |
| | % Tolerant Organisms | Increase |
| | % Dominant Taxon | Increase |
| **Feeding Measures** | | |
| | % Filterers | Variable |
| | % Grazers and Scrapers | Decrease |
| **Habitat Measures** | | |
| | Number of Clinger Taxa | Decrease |
| | % Clingers | Decrease |

Table 8. EPA's RBP potential benthic macroinvertebrate metrics and predicted responses to increasing stream perturbation.

| Metric Category | Metric | Predicted Response to increasing perturbation |
|---|---|---|
| **Richness Measures** | | |
| | Number of Pteronarcys species | Decrease |

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| | | |
|---|---|---|
| | Number of Diptera Taxa | Decrease |
| | Number of Chironomidae Taxa | Decrease |
| **Composition Measures** | | |
| | % Plecoptera | Decrease |
| | % Trichoptera | Decrease |
| | % Diptera | Increase |
| | % Chironomidae | Increase |
| | % Tribe Tanytarsini | Decrease |
| | % Other Diptera and non-insects | Increase |
| | % Corbicula | Increase |
| | % Oligochaeta | Variable |
| **Tolerance/Intolerance Measures** | | |
| | Number of Intolerant Snail and Mussel Species | Decrease |
| | % Sediment Tolerant Organisms | Increase |
| | Hilsenhoff Biotic Index | Increase |
| | Florida Index | Decrease |
| | % Hydropsychidae or Trichoptera | Increase |
| **Feeding Measures** | | |
| | % Omnivores and Scavengers | Increase |
| | % Collector Feeders of Coarse Particulate Organic Matter and Fine Particulate Organic Matter. | Variable |
| | % Gatherers | Variable |
| | % Predators | Variable |
| | % Shredders | Decrease |
| **Life Cycle Measures** | % Multivoltine | Increase |
| | % Univoltine | Decrease |

## FISH COMMUNITY ASSESSMENT

FIELD METHODS

Sample reaches should be consistently selected based on a fixed-distance designation or a proportional-distance designation at least 100 meters upstream from human disturbances and major tributary influences.

Block nets should be used to isolate the fish community to be sampled.

Fish less than 20 millimeters in length should not be included in the sample because of seasonal skewing effects on data, unreliable capture of such small individuals, age/size class ecological function differences, and difficulty of identification.

Backpack electrofishing units, tote barge electrofishing units, and seines are recommended sampling equipment. Electrofishing is conducted in an upstream direction and seining is conducted in a downstream direction.

All rare, threatened, endangered or other species in need of conservation should be identified and released in the field. Photographs may be taken to aid in identification of these species.

Field Preservation

   10% Buffered Formalin Solution

LABORATORY METHODS

Laboratory Preservation

10% Buffered Formalin Solution

Level of Taxonomic Identification

Species

DATA ANALYSIS AND INTERPRETATION

The Index of Biological Integrity (IBI) is recommended for analysis of fish communities. Figure 1generalizes the development of a fish IBI adapted for regional or local fish assemblages. The original metrics used in the IBI of Karr et al. (1986), and various potential other metrics, are listed in Table 9.

Figure 1. Sequential process of IBI development, calculation, and interpretation of the fish Index of Biological Integrity. Taken from EPA RPB second edition (Barbour, Gerritsen et al. 1999).
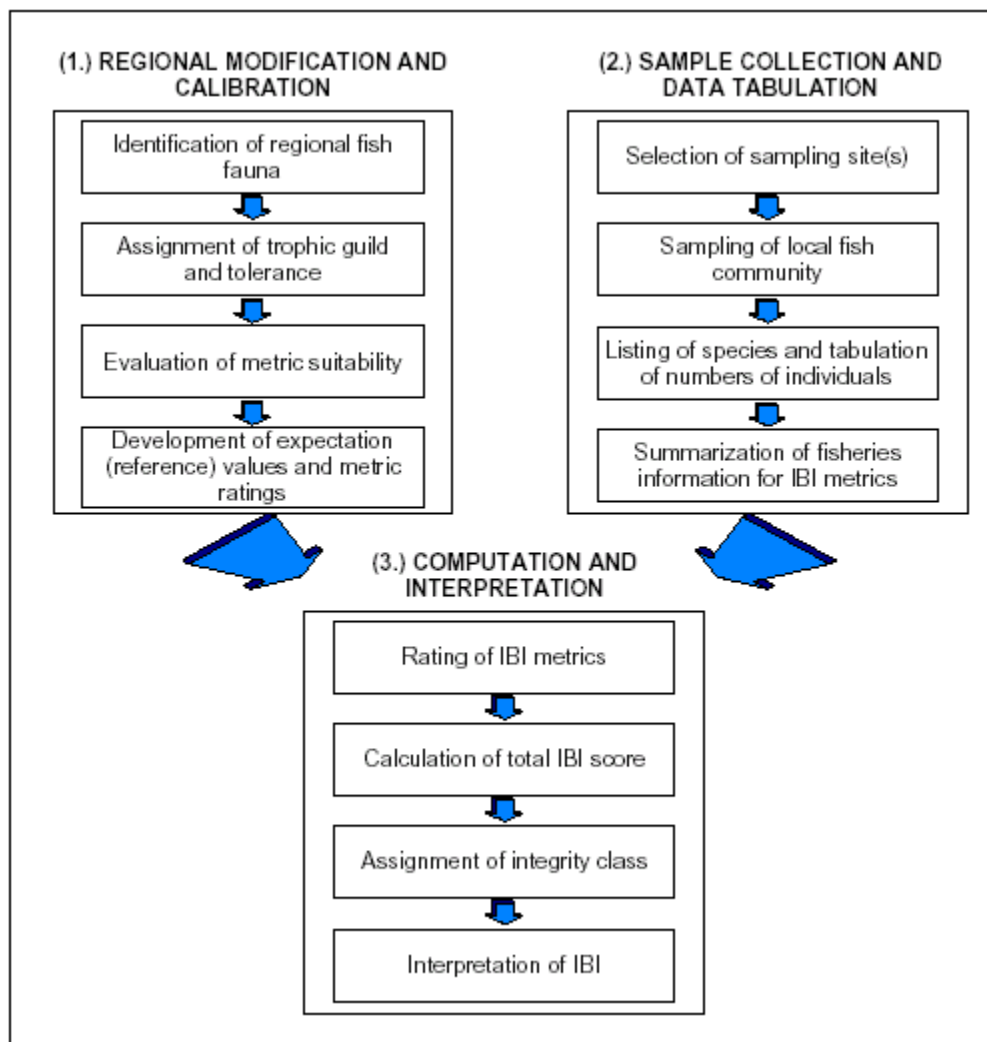
Table 9. Fish Index of Biological Integrity original metrics and alternative metrics developed for various regions of North America. Taken from EPA RPB second edition (Barbour, Gerritsen et al. 1999).

| Alternative IBI Metrics | Midwestern United States | Central Appalachians | Sacramento-San Joaquin | Colorado Front Range | Western Oregon | Ohio | Ohio Headwater Sites | Northeastern United States | Ontario | Central Corn Belt Plain | Wisconsin-Warmwater | Wisconsin-Coldwater | Maryland Coastal Plain | Maryland Non-Tidal |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **1. Total Number of Species** | X | X | X | X | | | | X | | X | | | X | X |
| #native fish species | | | | | | X | X | X | X | | X | | | |
| # salmonid age classes[b] | | | | X | X | | | | | | | | | |
| **2. Number of Darter Species** | X | X | | X | | X | | | | X | X | | | |
| # sculpin species | | | | | X | | | | | | | | | |
| # benthic insectivore species | | | | | | | | X | | | | | | |
| # darter and sculpin species | | | | | | | X | | | | | | | |
| # darter, sculpin, and madtom species | | | | | | | | | | | X | | | |
| # salmonid juveniles (individuals)[b] | | | X | | X | | | X | | | | | | |
| % round-bodied suckers | | | | | | | X[c] | | | | | | | |
| # sculpins (individuals) | | | X | | | | | | | | | | | |
| # benthic species | | | | | | | | | | | | | X | X |
| **3. Number of Sunfish Species** | X | | | X | | X | | | | X | X | | | |
| # cyprinid species | | | | | X | | | | | | | | | |
| # water column species | | | | | | | | X | | | | | | |
| # sunfish and trout species | | | | | | | | | X | | | | | |
| # salmonid species | | | X | | | | | | | X | | | | |
| # headwater species | | | | | | | X | | | | | | | |
| % headwater species | | | | | | | X | | | X | | | | |
| **4. Number of Sucker Species** | X | | | | X | X | | X | | X | X | | | |
| # adult trout species[b] | | | X | | X | | | | | | | | | |
| # minnow species | | | | X | | | X | | | X | | | | |
| # sucker and catfish species | | | | | | | | | X | | | | | |
| **5. Number of Intolerant Species** | X | | | X | X | X | | X | | | X | X | X | X |
| # sensitive species | | | | | | | X | | | X | | | | |
| # amphibian species | | | X | | | | | | | | | | | |
| presence of brook trout | | | | | | | | | X | | | | | |
| % stenothermal cool and cold water species | | | | | | | | | | | | X | | |
| % of salmonid ind. as brook trout | | | | | | | | | | | | X | | |
| **6. % Green Sunfish** | X | | | | | | | | | | | | | |
| % common carp | | | | | X | | | | | | | | | |
| % white sucker | | | | X | | | | | X | | | | | |
| % tolerant species | | | | | | X | X | | | X | X | X | X | X |
| % creek chub | | X | | | | | | | | | | | | |
| % dace species | | | | | | | | | X | | | | | |
| % eastern mudminnow | | | | | | | | | | | | | X | |

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Table 10. Continuation of Table 25.

| Alternative IBI Metrics | Midwestern United States | Central Appalachians | Sacramento-San Joaquin | Colorado Front Range | Western Oregon Ohio | Ohio Headwater Sites | Northeastern United States | Ontario | Central Corn Belt Plain | Wisconsin–Warmwater | Wisconsin–Coldwater | Maryland Coastal Plain | Maryland Non-Tidal |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 7. % Omnivores | X | | | X | | X | X | X | X | X | | | |
| % generalist feeders | | X | | | | | | | | | | | |
| % generalists, omnivores, and invertivores | | | | | | | | | | | | | X |
| 8. % Insectivorous Cyprinids | X | | | | | | | | | | | X | |
| % insectivores | | | | | X | | X | | X | X | | X | X[e] |
| % specialized insectivores | | X | | X | | | | | | | | | |
| # juvenile trout | | | X | | | | | | | | | | |
| % insectivorous species | | | | | | X | X | | | | | | |
| 9. % Top Carnivores | X | | | | | X | X | | X | X | X | | |
| % catchable salmonids | | | | | X | | | | | | | | |
| % catchable trout | | | X | | | | | | | | | | |
| % pioneering species | | | | | | | X | | X | | | X | |
| Density catchable wild trout | | | X | | | | | | | | | | |
| 10. Number of Individuals (or catch per effort) | X | X | X | X | X | X[d] | X[d] | | X | X | X[d] | X | |
| Density of individuals | | | | | | | | X | | | | | X |
| % abundance of dominant species | | | | | | | | | | | | X | X |
| Biomass (per m²) | | | | | | | | | | | | | X[f] |
| 11. % Hybrids | X | | | | | | X | | | | | | |
| % introduced species | | | | X | X | | | | | | | | |
| % simple lithophills | | | | | X | | | | X | X | | | X |
| # simple lithophills species | | | | | | | X | | | | | | |
| % native species | | | X | | | | | | | | | | |
| % native wild individuals | | | X | | | | | | | | | | |
| % silt-intolerant spawners | | | | | | | | | | | | X | |
| 12. % Diseased Individuals (deformities, eroded fins, lesions, and tumors) | X | X | | X | X | X | X | X | X | X | X | X | X |

Note: X = metric used in region. Many of these variations are applicable elsewhere.

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**PERIPHYTON**

Two protocols are presented in EPA's RBP. The first is a species composition and biomass method performed in a laboratory, and the second is a field-based rapid survey without the need of a high level of taxonomic expertise. The two methods may be used in conjunction.

STANDARD LABORATORY-BASED APPROACH

Field Methods

Multihabitat sampling or single habitat sampling may be used. Multihabitat sampling is recommended for characterizing a single stream reach. Single habitat sampling is described as being the preferred method for comparison of different stream reaches. The single habitat approach should still be based on a stream reach, not a single location in a stream. Artificial substrates may be used as conditions dictate, such as in non-wadeable streams.

Multihabitat Field Sampling

Algae are collected from all available habitat types. A single composite sample is collected to characterize the algae assemblage at a reach. 5-milliliter subsamples from each habitat may be used for the reach composite sample. Sampling consists of placing substrates, including woody debris, into a bucket and scrubbing the organisms free of the substrate. For large substrates that cannot be scrubbed in a bucket, a piece of PVC pipe with a neoprene collar fitted to one end is placed on the substrate and the area inside is scrubbed with a brush. Water is then removed from the PVC ring with a pipette and placed into a sample container.

Single Habitat Field Sampling

Algae should be collected from riffles and runs with cobble substrate if possible, if not samples should be taken from any hard substrates available. Phytoplankton may be a reasonable alternative in low gradient streams lacking solid substrates. Several subsamples are collected from the single habitat type and composited.

Artificial Substrate Field Sampling

Floating or benthic periphytometers may be used with substrates consisting of glass slides, glass rods, clay tiles, Plexiglas plates or similar substrates. A minimum of 3 replicates should be placed at each sample reach. Two to four weeks should be allowed for periphyton to colonize the substrates. Samples may be composited or analyzed individually.

Level of Taxonomic Identification

Lowest possible level and generic.

Field Preservation

Lugol's (IKI) solution

FIELD-BASED RAPID PERIPHYTON SURVEY

This approach is a semi-quantitative rapid periphyton survey of algal biomass and taxonomic composition. Three transects are established across a habitat, usually riffle or run with water clarity such that algal accumulation can be observed readily. Three locations along each transect, *e.g.*, right bank, middle, and left bank, are sampled. At each sample location a viewing bucket (≥0.5 m diameter) containing a grid of 50

dots, is immersed into the water. First, the number of dots that occur over macroalgae where no substrate can be seen are counted. Second, the type of algae at each dot is also recorded. The number of dots under which suitable substrata available for macroalgal accumulation is recorded and the thickness (density) of macroalgal accumulation is recorded according to a scale ranging from 0 to 5.

LABORATORY METHODS

Non-Diatom Algae Relative Abundance and Taxa Richness.

Samples are homogenized using tissue homogenizer or blender. Pipettes are used for sampling the homogenized samples for identification. 300 algal cells units are identified under 400× magnification.

Diatom Relative Abundance and Taxa Richness

Subsamples of at least 5-10 concentrated preserved samples are identified under oil immersion at 1000× magnification. A count of 600 valves equaling 300 cells should be made until 10 valves each of 10 species have been identified at minimum.

Periphyton Biomass

Chlorophyll $a$, ash-free dry weight, cell densities per $cm^2$, and biovolume per $cm^2$ may be used for rapid bioassessment of algal communities.

Laboratory Preservation

Lugol's (IKI) solution

Diatoms should be cleared using concentrated acid oxidation using nitric or sulfuric acid, or hydrogen dioxide.

Subsampling

In both non-diatom and diatom relative abundance and taxa richness, 300 cells should be counted.

DATA ANALYSIS AND INTERPRETATION

Algal Metrics used in Rapid Bioassessment

Metrics of Biotic Integrity
1. Species richness – diatoms, soft algae, or both
2. Total Number of Genera – diatoms, soft algae, or both
3. Total Number of Divisions
4. Shannon's Diversity Index
5. Percent Community Similarity
6. Pollution Tolerance Index for Diatoms
7. Percent Sensitive Diatoms
8. Percent *Achnanthes minutissima*
9. Percent Live Diatoms

Diagnostic Metrics that Infer Ecological Conditions
1. Percent Aberrant Diatoms
2. Percent Motile Diatoms, *e.g.*, *Navicula + Nitzchia + Surirella*
3. Simple Diagnostic Metrics - % relative abundance of species that have environmental optima in extreme environmental conditions.
        % acidobiontic + % acidophilic

% alkalibiontic + % alkaliphilic
% halophilic
% mesosaprobic + % oligosaprobic + % saprophilic
% eutrophic

4. Inferred Ecological Conditions with Simple Autecological Indices (SAI)
    PH Spectrum
    Nutrient (N and P) Spectrum
    Halobion Spectrum – based on chloride concentrations or conductivity
    Saprobien System –based on organic pollution

5. Inferred Ecological Conditions with Weighted Average Indices – based on specific ecological optima for algae

6. Impairment of Ecological Conditions – inferred from by calculating the deviation between inferred environmental conditions at a test site and at a reference site.

Algal Biomass

Repeated measures of algal biomass allow for the mean and maximum benthic chlorophyll *a* to be used in defining the trophic status of a stream, *e.g.*, oligotrophic, eutrophic, etc.

Information from the viewing bucket rapid assessment method should be expressed as the mean density of each type of macroalgae on suitable substrates and the maximum density of each type of microalgae on suitable substrates.

## PHYSICAL HABITAT ASSESSMENT

The EPA RBP habitat assessment is a visually based estimate of the quality of ten basic and important habitat features important to the ecological function of a stream.  Taking into account the fundamental differences in the structure of high and low gradient streams several of the parameters are scored differently based on high or low gradient status of the stream.  The parameters are scored subjectively on either a scale of 0 to 20 or 0 to 10 with the scores divided into four classes defined as optimal, suboptimal, marginal, or poor.  Listed below are 13 parameters whose quality is visually estimated.

1) Epifaunal Substrate/Available cover
2) Substrate Embeddedness
3) Pool Substrate Characterization
4) Velocity/Depth Combinations
5) Pool Variability
6) Sediment Deposition
7) Channel Flow Status
8) Channel Alteration
9) Frequency of Riffles or Bends
10) Channel Sinuosity
11) Bank Stability
12) Bank Vegetative Protection
13) Riparian Vegetative Zone Width

In some instances it may be desired to include some quantitative measurements and it is recommended in EPA RBP's to utilize EPA's EMAP protocols for quantitative habitat assessments.

## REFERENCE SITE SELECTION

Reference conditions should be selected based on the goal of the monitoring effort.  For some monitoring objectives (e.g. short-term impact studies), site specific or paired watershed reference conditions may be appropriate.  For others, a regional approach may be more appropriate.  No additional guidance given.

### III. UNITED STATES GEOLOGICAL SURVEY: NATIONAL WATER-QUALITY ASSESSMENT PROGRAM (NAWQA).

The NAWQA program (Fitzpatrick, Waite et al. 1998; Moulton, Kennen et al. 2002)was implemented in 1991 as a national effort to provide data in support of national, regional, and local policy and management of water quality.  The protocols utilized by NAWQA are supportive of biological assessment and long-term trend monitoring of aquatic resources affected by the quality of water.

**SAMPLING REACH CONSIDERATIONS**

The recommended reach length for wadeable streams is 150 to 300 meters depending on stream width, depth, and habitat disturbances.  For non-wadeable streams and rivers, sampling reach lengths between 500 and 1000 meters are recommended.

**MACROINVERTEBRATE COMMUNITY ASSESSMENT**

<u>FIELD METHODS AND INDEX PERIOD</u>

Semi-Quantitative Sampling

> A series of discrete semi-quantitative samples are taken from Richest-Targeted Habitats (RTH). RTHs are microhabitats of high macroinvertebrate abundance and taxa richness such as cobble riffles, woody debris snags, or the bases of emergent macrophytes.  The RTH samples are combined into a single composite sample for the reach.  No guidelines are given in the protocols for the number of samples to be taken at a particular site or habitat.

> Equipment that may be used, depending on the characteristics of the sample reach is:

> Slack Sampler with 500-micron screen, and 0.25 meters squared template area.

> For fine substrates such as sand and silt, Ekman or Petite Ponar grab samplers may be used. Screens on the samplers must be 500 microns.

Qualitative Sampling

> Discrete samples are collected from each habitat type in the sampling reach and are referred to as Qualitative Multi Habitat (QMH) samples, which are combined into a single composite qualitative sample for the reach.

> D-frame kick net with 500-micron mesh netting

> Handpicking selected large substrates, *e.g.*, large cobble, leaf packs, and root wads.

> Grab samplers such as Ekman or Petite Ponar with 500-micron screens for fine substrates.

> 1 hour of sampling time is allocated to QMH at the reach, dividing time proportionally between the different habitat types within the reach.

Index Period

> No guidelines presented.

Field Preservation

10% Formalin solution

Dependent upon study objectives.

## FISH COMMUNITY ASSESSMENT

### FIELD METHODS

Electrofishing may be conducted with a boat-mounted, towed, or backpack electrofishing unit depending on stream size and depth.  Two passes are made through the sample reach.  Data for each pass is recorded separately.

Block nets may be used, but are not required for NAWQA studies.

Seining is conducted in all habitat types, with all types of seines having ¼ inch mesh.  Common sense seines, collection, and bag seines of different lengths and heights may be used depending on the situation in the reach.  3 seine hauls per habitat.  Three hauls from a habitat type are combined as a composite sample for that habitat type.

Field Preservation

10% Formalin solution

### DATA ANALYSIS AND INTERPRETATION

Dependent upon study objectives.

## ALGAL COMMUNITY ASSESSMENT

### QUANTITATIVE

Select a sampling method that allows for the total area sampled from the substrate to be calculated.

Richest Target Habitat (RTH) - 3 subsamples

1) chlorophyll *a*
2) ash-free dry mass (AFDM)
3) taxa richness, cell density, relative abundance, biovolume of algal species

Depositional Targeted Habitat  (DTH) - 3 subsamples

1) chlorophyll *a*
2) ash-free dry mass (AFDM)
3) taxa richness, cell density, relative abundance, biovolume of algal species

Phytoplankton (PHY) - 3 subsamples

1) chlorophyll *a*
2) particulate organic carbon (POC
3) taxa richness, cell density, relative abundance, biovolume of algal species

<u>QUALITATIVE</u>

Qualitative Multi Habitat (QMH)

Scraping, brushing, and siphoning periphyton from submerged substrates in five different habitats.  The five samples are composited.

<u>MACROALGAE</u>

Qualitative samples of large filamentous algae are collected from each habitat present.

<u>DATA ANALYSIS AND INTERPRETATION</u>

Dependent upon study objectives.

**PHYSICAL HABITAT ASSESSMENT**

NAWQA protocols integrate physical habitat data taken at basin, segment, reach, and microhabitat scales.  Basin and segment scale data are collected using GIS databases or calculated manually from 7.5 minute topographic maps.  Reach and microhabitat scale data are collected using field-based surveys on site utilizing a transect system.  The reach is divided into ten evenly spaced segments between eleven transect lines.

<u>BASIN SCALE DATA</u>

| | |
|---|---|
| Total Drainage Area | Minimum Elevation in Basin |
| Average Annual Runoff | Maximum Elevation in Basin |
| Average Annual Air Temperature | Basin Relief Ratio |
| Average Annual Precipitation | Drainage Shape |
| Average Annual Class A Pan Evaporation | Stream Length |
| Basin Length | Cumulative Perennial Stream Length |
| Drainage Density | Drainage Texture |
| Entire Stream Gradient | Estimated Flow Characteristics |
| Land Use/Land Cover | Soils |
| Geology | Physiography |
| Ecoregions | Potential Natural Vegetation |
| Land-resource Areas | Wetlands |

<u>SEGMENT SCALE DATA</u>

| | |
|---|---|
| Location of Segment Boundaries | Segment Length |
| Curvilinear Channel Length and | Upstream and Downstream Elevation |
|    Distance to Reference Location | Sinuosity |
| Segment Gradient | Water Management Feature |
| Stream Order | Valley Sideslope Gradient |

<u>REACH SCALE DATA</u>

| | |
|---|---|
| Discharge | Channel Modifications |
| Curvilinear Reach Length | Surface Water Gradient |
| Geomorphic Channel Units | |

<u>TRANSECT BASED INFORMATION</u>

| | |
|---|---|
| Wetted Channel Width | Bankfull Channel Width |
| Channel Features (bars, islands, shelves, etc.) | Canopy Angles |

| Riparian Canopy Closure | Dominant Riparian Land Use/Land Cover |
|---|---|
| Bank Angle | Bank Height |
| Bank Substrate | Bank Vegetative Cover |
| Bank Erosion | Habitat Cover Features (presence/absence) |
| Thalweg Depth | Depth |
| Velocity | Dominant Bed Substrate |
| Embeddedness | Silt Presence/Absence |

## DATA ANALYSIS AND INTERPRETATION

Table 11. USGS Bank Stability Index an indicator of overall bank condition.

| Bank Characteristic | Measurement | Score |
|---|---|---|
| Bank Angle (degrees) | 0-30 | 1 |
| | 31-60 | 2 |
| | >60 | 3 |
| Vegetative Cover (%) | >80 | 1 |
| | 50-80 | 2 |
| | 20- <50 | 3 |
| | <20 | 4 |
| Bank Height (meters) | 0-1 | 1 |
| | 1.1-2 | 2 |
| | 2.1-3 | 3 |
| | 3.1-4 | 4 |
| | >4 | 5 |
| Substrate (category) | bedrock, artificial | 1 |
| | boulder, cobble | 2 |
| | silt | 5 |
| | sand | 8 |
| | gravel/sand | 10 |

| | Total Score | Interpretation |
|---|---|---|
| | 4-7 | Stable |
| | 8-10 | At risk |
| | 11-15 | Unstable |
| | 16-22 | Very unstable |

## RECOMMENDED STATISTICAL TECHNIQUES

Habitat data analysis in NAWQA studies is determined by the goal of the study. Below are some of the techniques recommended.

- Graphical plotting or correlation analyses of habitat variables with respect to response variables.
- Parametric: correlation analyses, analysis of variance (ANOVA)
- Non-parametric: Spearman rank correlations, Kruskal-Wallis test, Tukey standardized range test, and Wilcoxin sign-ranks
- Principal Component Analysis (PCA)
- Indirect Gradient Analysis: Detrended Correspondence Analysis (DCA)
- Direct Gradient Analysis: Canonical Correspondence Analysis (CCA)
    - Analysis of Variance (ANOVA)

## REFERENCE SITE SELECTION

No specific guidance given. However, NAWQA references Harrelson et al., who recommend the establishment of reference sites based on representative watershed types, historical conditions, and best professional judgment (Harrelson, Rawlins et al. 1994).

## IV. UNITED STATES FOREST SERVICE PROTOCOLS.

The protocols presented by USFS (Plats, Megahan et al. 1983) have the primary purpose of evaluating conditions related to salmon fish production. Habitat, invertebrate, and fish sampling and data interpretation are all aimed at assessing a stream reach's ability to promote and sustain salmonid growth and recruitment.

## SAMPLING REACH CONSIDERATIONS

Transect systems are recommended for use in physical habitat assessment. The objectives of the study determine what type of transect system is used. USFS recommends the transect cluster system using one of three approaches.

> 1. Multiple Transect – Determine the number of transects required to detect statistical differences, then randomly select transects from the stream reach under study.
>
> 2. Multiple Station – Randomly select sample points in the reach under study, then group the required number of transects to obtain statistical significance around these points.
>
> 3. Stratified Random – The required assumption is that a good deal of information on the reach already exists (*i.e.* knowledge of the available habitats and their distribution). The stratified random design allows the investigator to target and intensively sample more complex habitats while reducing sampling in less complex and biologically important habitats.

## MACROINVERTEBRATE COMMUNITY ASSESSMENT

Sample Frequency and Size

USFS recommends a minimum of one sample per season and three replicates per habitat type. However, it is recommended that the minimum sample size needed to detect statistically significant differences should be calculated from preliminary field data for each site.

Semi-Quantitative Sampling Equipment

Hester-Dendy
Modified Hess
Surber

Field Preservation

70% ethanol or 5% formalin solution

Level of Taxonomic Identification

Dependent on project resources and objectives, but generally genus or species level.

Data Interpretation and Analysis

| | |
|---|---|
| Species Abundance | Biotic Condition Index |
| Taxa Richness | Chandler Biotic Score |
| Shannon-Wiener Diversity | |

## FISH COMMUNITY ASSESSMENT

The fish community assessment methods presented by USFS are designed to provide population estimates of salmonids in streams, but may be used to estimate populations of any fish species of interest. Depletion electrofishing is recommended for determining standing stocks of fish species per area of stream. Sodium cyanide, rotenone, direct underwater counting, and explosives are also presented as sampling alternatives. Several quantitative methods for determining population size are recommended. Two basic examples calculated from seine haul or electrofishing data are presented below.

Depletion sampling and estimating population size:

Two-Step Method – Block nets are used to isolate the sampling reach. Two electrofishing passes are made with numbers per species recorded for each pass individually. Population size (N) for the sample reach for each species or all species is estimated with the following formula.

$$N \text{ (est)} = \frac{(N \text{ first pass})^2}{(N \text{ first pass} - N \text{ second pass})}$$

Four-Step Zippin Method – Block nets are used to isolate the sample reach. Four electrofishing or seine haul passes are made. More or less than four passes may be used.

The Zippin method is a maximum likelihood model using probabilities to determine fish population size (N) within a sample reach. USFS recommends four electrofishing passes for use with the Zippin method. Zippin population size is estimated as follows.

$$N \text{ (est.)} = T/Q$$

Where: T = total number or fish

Q = sampling fraction (determined graphically)

Graphical Determination of Q:
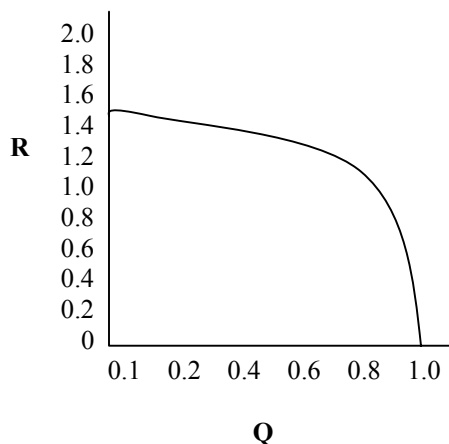


$$\text{Where } R = \frac{\sum_{i=1}^{k}(i-1)U_i}{T}$$

K = number of removals
$U_i$ = number of fish collected in $i$th removal

**PHYSICAL HABITAT ASSESSEMENT**

WATER COLUMN MEASUREMENTS

| Wetted Stream Width | Average Depth |
| Stream Shore Water Depth (esp. undercut banks) | Pool-riffle ratio |
| Pool, riffle, run widths and lengths | Pool quality - index |
| Pool Feature (feature forming the pool) | Channel bank angle |

STREAM BOTTOM MEASUREMENTS

| Channel elevation | Channel gradient |
| Channel sinuosity | Stream channel substrate |
| Sedimentation | Erosion and deposition |
| Channel debris and sediment storage | Stream order |

RIPARIAN ZONE MEASUREMENTS

| Streamside cover | Vegetation use by animals |
| Herbage production and utilization – electronic capacitance meter reading | Habitat type – stream bank |
| | Vegetation overhang |

**REFERENCE SITE SELECTION**

No guidance given.

# SUMMARY OF BIOLOGICAL ASSESSMENT METHODS USED BY SELECT STATES OUTSIDE USEPA REGION 7

# I. STATE OF ARKANSAS

The Arkansas Department of Environmental Quality biological monitoring program utilizes macroinvertebrate and fish communities.  Fish communities are evaluated following methods contained in USEPA's Technical Support Manual: Waterbody Surveys and Assessments for Conducting Use Attainability Analysis (USEPA 1983) with an emphasis on the management of game fish. Macroinvertebrate communities are evaluated following USEPA's Rapid Bioassessment Protocols for Use in Wadeable Streams and Rivers (Plafkin, Barbour et al. 1989). Other details of methodology are outlined in state documents (7/1/1994; Arkansas Game and Fish Commission and USDA Forest Service 1994).

## SAMPLING REACH CONSIDERATIONS

No related material.

## MACROINVERTEBRATE COMMUNITY ASSESSMENT

### FIELD METHODS AND INDEX PERIOD

A D-frame net with a width of 0.3 meters and a mesh size of 500 microns is used for the collection of macroinvertebrates.  Because Arkansas' topography results in two very different general habitat conditions for streams, Ozark mountain high gradient streams and lowland low gradient streams, a different method is utilized for each type of stream. Macroinvertebrates are collected in both during the spring (April through mid-June) and fall (September through October).  It appears that a single composite sample is collected at each site and consists of 20 one-meter kick samples.

Mountain Regions –

Traveling kick method.  Five-minute kick samples along diagonal transect.  Two riffles sampled at each site.

Low Gradient Streams –

Twenty one-meter long kick samples taken to equal approximately 6 square meters of sampled substrate.  Habitats are sampled in proportion to their area in sample reach.

Specimens preserved in the field with 70% ETOH

### LABORATORY METHODS

Subsampling

4-inch diameter ring randomly tossed into tray containing samples.  Invertebrates collected from ring.  Method repeated until a minimum of 95 organisms has been sampled.

Level of Taxonomic Identification

No related material.

### DATA ANALYSIS AND INTERPRETATION

Table 12. Metrics used by Arkansas Department of Environmental Quality for the interpretation of macroinvertebrate community data.

| Data Category | Metric | Expected metric response to increasing perturbation |
|---|---|---|
| Taxa Richness | Number of taxa | Decrease |
| | Number of EPT taxa | Decrease |
| | Number of Ephemeroptera taxa | Decrease |
| | Number of Plecoptera taxa | Decrease |
| | Number of Trichoptera taxa | Decrease |
| | Number of Coleoptera taxa | Decrease |
| | Number of Diptera Taxa | Decrease |
| Community Composition Measures | Shannon-Weiner Index | Decrease |
| | % dominant taxon | Increase |
| | % EPT | Decrease |
| | % Ephemeroptera | Decrease |
| | % Plecoptera | Decrease |
| | % Trichoptera | Decrease |
| | % Diptera | Increase |
| | % Chironomidae | Increase |
| | % Amphipoda | Decrease |
| | % Isopoda | Increase |
| Tolerance Measures | Hilsenhoff Biotic Index | Increase |
| | Number of Intolerant taxa | Decrease |
| | % Tolerant taxa | Decrease |
| Trophic Measures | % Shredders | Decrease |
| | % Collectors | Variable |
| | % Filterers | Decrease |
| | % Scrapers | Decrease |
| | % Predator | Variable |

**FISH COMMUNITY ASSESSMENT**

ADEQ uses the methods and protocols presented in USEPA's Technical Support Manual: Waterbody Surveys and Assessments for Conducting Use Attainability Analysis (USEPA 1983).

Arkansas Game and Fish Commission uses a variety of fish sampling techniques utilizing both quantitative and qualitative sampling. Electrofishing is conducted with either a backpack unit or an electrofishing boat unit. Depletion, mark-recapture, and catch per unit effort (CPUE) data are collected depending on the management goals of the sampling effort. Rotenone sampling is conducted primarily in areas such as bayous where current is slow and water is very turbid. Explosives are sometimes used because of its effectiveness in collecting large fish. Seines, trawls, and gill, trammel, hoop, and trap nets are used to collect qualitative, mark-recapture, and CPUE data.

**PHYSICAL HABITAT ASSESSMENT**

The physical habitat assessment methods used by ADEQ are taken from USEPA's Rapid Bioassessment Protocols for Use in Wadeable Streams and Rivers (Plafkin, Barbour et al. 1989).  It consists of subjectively scoring several categories of instream and riparian habitat parameters on a 0 to 20 scale. Habitat quality is rated according to four qualitative condition categories defined as poor, marginal, suboptimal, and optimal.

**REFERENCE SITE SELECTION**

No related material.

**BIOLOGICAL CRITERIA**

<u>Narrative</u>

Biological Integrity - All waters with specifically designated Fisheries uses must demonstrate aquatic life communities, which are similar in variety and abundance to least-disturbed waters within the same ecoregion and with similar hydrologic conditions. Measurements of biological integrity should include fish community structure and other associated aquatic life *e.g.*, macroinvertebrates, periphyton, plankton, etc. Measurements should be extensive and timely in order to compensate for the seasonal and natural variability of aquatic life communities. A distinguishable alteration of the abundance or variety of the aquatic life community constitutes a violation of these water quality standards.

<u>Numeric</u>

None

## II. STATE OF INDIANA

Sampling protocols are presented in a variety of state documents (Indiana Department of Environmental Management 2001; Indiana Department of Environmental Management 2001; Dofour Consulting No date given).

**SAMPLING REACH CONSIDERATIONS**

Avoid areas of obvious degradation, bridges provide convenient access. For some studies sample sites are chosen randomly and are sampled regardless of condition.

**MACROINVERTEBRATE COMMUNITY ASSESSMENT**

<u>FIELD METHODS AND INDEX PERIOD</u>

Indiana Department of Environmental Management only indicates that several USEPA Rapid Bioassessment Protocol methods are used (Indiana Department of Environmental Management 2001; 2001). The sampling period for macroinvertebrates extends from July to October. The number of macroinvertebrate samples collected at each site was not specified but each site is visited on a rotational basis such that sampling occurs no more than once ever other year.

<u>LABORATORY METHODS</u>

      Subsampling

            100 organism subsample

      Level of Taxonomic Identification

            Family

<u>DATA ANALYSIS AND INTERPRETATION</u>

Average multi-metric score compared to reference conditions. Metrics used not listed.

**FISH COMMUNITY ASSESSMENT**

<u>FIELD METHODS</u>

No related material except that fish are sampled once per site and sampling is done on a rotational watershed basis such that sites are visited once every two to five years or longer.

<u>LABORATORY METHODS</u>

No related material.

<u>DATA ANALYSIS AND INTERPRETATION</u>

Fish data are analyzed using the Index of Biological Integrity (IBI). The IBI is plotted against Ohio EPA's Qualitative Habitat Evaluation Index (QHEI) to determine relationships between habitat and fish community characteristics.

Table 13. Fish Index of Biological Integrity metrics used by the Indiana Department of Environmental Management.

| Fish IBI metrics used by IDEM | |
|---|---|
| Total Number of Species | Percent omnivore species |
| Number of darter, madtom, and sculpin species | Percent insectivore species |
| Number of darter species | Percent pioneer species |
| Percent headwater species | Percent carnivore species |
| Number of sunfish species | Catch per unit effort/number of individuals |
| Number of minnow species | Percent simple lithophilic species |
| Number of sucker species | Percent DELT anomalies |
| Number of sensitive species | Percent tolerant species |
| Number of salmonid species | Percent large river species |
| Number round-bodied sucker species | Number of centrarchid species |
| Catch per unit effort/number of individuals – gizzard shad | |

## PHYSICAL HABITAT ASSESSMENT

Follows Ohio EPA's Qualitative Habitat Evaluation Index (QHEI).

## REFERENCE SITE SELECTION

Reference conditions determined using regional site aggregation methods and professional judgment. Deviations from central tendency in multimetric biological indices and Ohio EPA's QHEI are taken into consideration.

## BIOLOGICAL CRITERIA

Under Development.

# III. STATE OF MINNESOTA

The macroinvertebrate biological assessment SOPs of Minnesota Pollution Control Agency concentrate on wetlands. The wetlands SOPs also include assessment and calculation of indices based on vegetative sampling. Stream macroinvertebrate protocols are under development and not currently available. However, fish SOPs are detailed and data analysis of fish data in the form of IBIs is separated into two protocols developed for cool-water streams of the upper Mississippi River basin and cool-water streams of the St. Croix River basin. Methodologies are documented in a variety of references (1994; Niemela and Feist 2000; Gernes and Helgen 2002; Niemela and Feist 2002; Genet and Chirhart 2004; Minnesota Pollution Control Agency No date given; Minnesota Pollution Control Agency No date given; Minnesota Pollution Control Agency No date given).

## SAMPLING REACH CONSIDERATIONS

Sampling reach for fish should be 35 times the mean stream width.

## MACROINVERTEBRATE COMMUNITY ASSESSMENT

### FIELD METHODS AND INDEX PERIOD

Standard No. 30 sieve.

D-frame net with 600-micron mesh. Two samples are taken within same general location of nearshore emergent vegetation by sweeping action of the net 3-5 times. The two samples are composited.

Activity trap samplers are used to sample invertebrates that are active swimmers or night-active predators. This is a device constructed from clear 2-liter beverage containers by cutting the top of the container off and inverting it into the body of the container. Ten activity traps are deployed in 5 pairs 3-4 meters apart in wetlands near shoreline emergent vegetation and left for two nights. The samples are composited. A sampling period of June to early July is preferred, but sampling could be performed earlier if spring temperatures permit.

Field Preservation

        80% ETOH

### LABORATORY METHODS

Level of Taxonomic Identification

        Typically lowest level, but most commonly genus level.

Laboratory Preservation

        80% ETOH

### DATA ANALYSIS AND INTERPRETATION

        Stream macroinvertebrate IBI, are in development and may be similar to those proposed for the Upper Mississippi River basin in Minnesota Table 14.

        Wetland macroinvertebrate IBI metrics are listed in Table 15.

Table 14. Scoring criteria for the three separate M-IBIs developed for the Upper Mississippi River Basin in Minnesota (modified from Genet and Chirhart 2004).

| Metric | Range | Response to disturbance | Score | | |
|---|---|---|---|---|---|
| | | | 0 | 2 | 4 |
| **Riffle/Run, < 500 mi$^2$** | | | | | |
| # Trichoptera Taxa | 1–15 | decrease | 0–4 | 5–8 | >8 |
| # Ephemeroptera + Plecoptera Taxa | 1–9 | decrease | 0–4 | 5–6 | >6 |
| # Diptera Taxa | 4–24 | decrease | 0–10 | 11–16 | >16 |
| # Orthocladiinae +Tanytarsini Taxa | 1–11 | decrease | 0–4 | 5–7 | >7 |
| # Intolerant Taxa | 0–14 | decrease | 0 | 1–4 | >4 |
| # Scraper Taxa | 0–13 | decrease | 0–4 | 5–7 | >7 |
| # Collector–Gatherer Taxa | 3–19 | decrease | 0–10 | 11–14 | >14 |
| % Trichoptera (excluding Hydropsychidae) | 0–22.2 | decrease | 0 | >0–3.3 >3.3 | |
| % Non–Insect | 2.8–76.2 | increase | >42.6 | >22.7–42 . | 6 0–22.7 |
| HBI | 4.77–7.67 | increase | >6.70 | >5.74–6.70 | <5.74 |
| **Glide/Pool, < 40 mi$^2$** | | | | | |
| POET | 1–16 | decrease | 0–6 | 7–11 | >11 |
| # Clinger Taxa | 0–11 | decrease | 0–4 | 5–7 | >7 |
| # Collector–Filterer Taxa | 1–8 | decrease | 0–3 | 4–6 | >6 |
| # Intolerant Taxa | 0–5 | decrease | 0–2 | 3 | >3 |
| % Dominant Taxon | 12.8–65.4 | increase | >47.8 | >30.3–47.8 | <30.3 |
| % Ephemeroptera | 0–50.3 | decrease | 0–5.9 | >5.9–22.8 | >22.8 |
| % Intolerant | 0–32.1 | decrease | 0–1 | >1–3.3 | >3.3 |
| % Tolerant | 28.2–95.1 | increase | >72.8 | >50.5–72.8 | 0–50.5 |
| % Trichoptera (excluding Hydropsychidae) | 0–8.4 | decrease | 0 | >0–1 | >1 |
| HBI | 4.85–8.65 | increase | >7.38 | >6.11–7.38 | <6.11 |
| **Glide/Pool, > 40 mi$^2$** | | | | | |
| % Coleoptera + Hemiptera | 0–38.4 | increase | >16.5 | >8.2–16.5 | 0–8.2 |
| # Gastropoda Taxa | 1–6 | decrease | 0–2 | 3–4 | >4 |
| # Non–Insect Taxa | 4–10 | decrease | 0–6 | 7–8 | >8 |
| % Caenidae | 0–43.2 | increase | >7 | >0–7 | 0 |
| % Oligochaeta | 0–10.6 | increase | >2.3 | >1.1–2.3 | 0–1.1 |
| % Crustacea + Mollusca | 0.6–94.6 | decrease | 0–26.2 | >26.2–51.7 | >51.7 |
| # Odonata + Trichoptera Taxa | 2–17 | decrease | 0–7 | 8–12 | >12 |

Table 15. Wetland invertebrate IBI metrics with criteria and score for Minnesota large depressional wetlands.

| Metric | Criteria | Score |
|---|---|---|
| Total invertebrate taxa | >51-77 | 5 |
| | >36-51 | 3 |
| | <21-36 | 1 |
| Odonata taxa | >4 | 5 |
| | 3-4 | 3 |
| Chironomid genera taxa | 14->21 | 5 |
| | 7-13 | 3 |
| | 0-6 | 1 |

| | | |
|---|---|---|
| Leech taxa | 5-9 | 5 |
| | 3-4 | 3 |
| | 0-2 | 1 |
| Snail taxa | 7-9 | 5 |
| | 4-6 | 3 |
| | 0-3 | 1 |
| ETSD Metric: number of mayfly genera, number of caddisfly genera, presence of fingernail clams, presence of dragonflies | >6-10 | 5 |
| | >3-6 | 3 |
| | 0-3 | 1 |
| Number of intolerant taxa | 5-7 | 5 |
| | 3-4 | 3 |
| | 0-2 | 1 |
| Proportion of tolerant taxa | 16-42% | 5 |
| | >42-69% | 3 |
| | >69% | 1 |
| Proportion of dominant 3 taxa | <34-54% | 5 |
| | >54-74% | 3 |
| | >74-94% | 1 |
| Proportion of Corixidae as beetles and bugs in activity traps | <33% | 5 |
| | 33-67% | 3 |
| | >67% | 1 |

## FISH COMMUNITY ASSESSMENT

### FIELD METHODS

Backpack electrofishing units used in streams with less than 8 meters mean stream width and watersheds less than 50 square miles.  A single run is conducted in an upstream direction.

Towed electrofishing units used in streams with a mean stream width greater than 8 meters and a watershed size of 50-500 square miles.  A single run is conducted in an upstream direction.

Mini-boom electrofishing unit is used in non-wadeable streams where accessibility for large electrofishing boats is not feasible or the stream is too small for such craft.  The mini-boom is an electrofishing unit set up in a small "jon" style boat.  A single run is conducted in a downstream direction.

Boom-shocker is used in large accessible rivers.  This is a large stable boat capable of carrying a three-person electrofishing crew.  Three runs are conducted in a downstream direction; one run on each bank of the river and one in the mid-channel.

Voucher specimens are retained.  Fish less than 25 mm in total length are not included in data.  Total length and minimum and maximum lengths are recorded for each species and the collective weight of each species measured obtained to the nearest 0.5 gram.


Field Preservation

    10% formalin solution


### LABORATORY METHODS

No related material.

DATA ANALYSIS AND INTERPRETATION

Minnesota has developed several fish IBIs for the upper Mississippi River basin and the St. Croix River basin. Various IBI metrics are applied in each basin depending on drainage size, creating tailored IBIs for these two major basins and for various drainage sizes within each basin. A major deviation in the Minnesota Pollution Control Agency's IBIs from most is that the scoring criteria for the metrics have a wider range of values, using a scoring system ranging from 0 to 10 instead of the usual 1, 3, and 5. The total IBI score range is 0 to 100. The tables on the following pages show generally how the MPCA fish IBIs metrics are scored and interpreted.

Table 16. Interpretation of MPCA fish IBI metrics total score.

| Total IBI Score | Biological Integrity Rating | Interpretation |
|---|---|---|
| 100-80 | Excellent | Comparable to minimal disturbance. |
| 79-60 | Good | Somewhat disturbed. |
| 59-40 | Fair | Decreased species richness, some signs of ecological imbalance. |
| 39-20 | Poor | Decreased species richness, growth and condition of fish depressed. |
| 19-0 | Very Poor | Fish community severely modified by human disturbance. |
| no score | | Few or no fish, impossible to calculate score. |

Table 17. Scoring criteria for the 7 metrics used to calculate the IBI for very small streams (< 5 mi$^2$ drainage area) in the Upper Mississippi River Basin of Minnesota*.

| | Scoring criteria | | | | |
|---|---|---|---|---|---|
| Metric | 10 | 7 | 5 | 2 | 0 |
| **Species richness and composition metrics** | | | | | |
| Total number of species | 9 or more | 7 or 8 | 5 or 6 | 3 or 4 | 0 - 2 |
| Number of wetland species** | 2 or more | | 1 | | 0 |
| Percent tolerant species*** | 0-80 | 81-85 | 86 - 90 | 90-95 | 96-100 |
| Percent dominant two species*** | 0-60 | 61-70 | 71 - 80 | 81-90 | 91-100 |
| **Trophic metrics** | | | | | |
| Number of invertivore species** | 2 or more | | 1 | | 0 |
| **Fish abundance and condition metrics** | | | | | |
| Number of fish per 100 meters | 2 | | 5 or more | | 0-4 |
| Percent DELT anomalies*** | 0-1 | | 2 or 3 | | 4 or more |

\* For very small streams the sum of the 7 metrics must be multiplied by 1.43 to obtain a final IBI score.
\*\*The metrics for number of wetland species, number of invertivore species, and number of fish per 100 meters do not include tolerant species.\*\*\*Round all percent metrics to the nearest 1 percent.

Table 18. Scoring criteria for the 10 metrics used to calculate the IBI for small streams (5 to 35 mi$^2$ drainage area) in the Upper Mississippi River Basin of Minnesota.

| | Scoring Criteria | | | | |
|---|---|---|---|---|---|
| Metric | 10 | 7 | 5 | 2 | 0 |
| **Species richness and composition metrics** | | | | | |
| Total number of species | 14 or more | 11-13 | 8-10 | 5-7 | 0-4 |
| Number of wetland species* | 3 or more | | 1 or 2 | | 0 |
| Number of minnow species* | 5 or more | 4 | 2 or 3 | 1 | 0 |
| Number of intolerant species | 2 or more | | 1 | | 0 |
| Percent tolerant species** | 0-40 | 41-55 | 56-70 | 71-85 | 86-100 |

| | | | | | |
|---|---|---|---|---|---|
| Percent dominant two species** | 0-52 | 53-64 | 65-76 | 77-88 | 89-100 |
| **Trophic and reproductive function metrics** | | | | | |
| Number of invertivore species* | 5 or more | 4 | 2 or 3 | 1 | 0 |
| Percent simple lithophils** | 49-100 | 37-48 | 25-36 | 13-24 | 0-12 |
| **Fish abundance and condition metrics** | | | | | |
| Number of fish per 100 meters* | 5 or more | | 0–4 | | |
| Percent DELT anomalies** | 0-1 | | 2 or 3 | 4 or more | |

\* Number of wetland species, number of minnow species, number of invertivore species, and number of fish per 100 meters metrics do not include tolerant species.

\*\* Round all percent metrics to the nearest 1 percent.


Table 19. Scoring criteria for the 10 metrics used to calculate the IBI for moderate size streams (35 to 200 mi$^2$ drainage area) in the Upper Mississippi River Basin of Minnesota.

| | Scoring Criteria | | | | |
|---|---|---|---|---|---|
| Metric | 10 | 7 | 5 | 2 | 0 |
| **Species richness and composition metrics** | | | | | |
| Total number of species | 20 or more | 16-19 | 12-15 | 8-11 | 0-7 |
| Number of darter, sculpin, and madtom species | 4 or more | 3 | 2 | 1 | 0 |
| Number of wetland species* | 3 or more | | 1 or 2 | | 0 |
| Number of intolerant species | 4 or more | 3 | 2 | 1 | 0 |
| Percent tolerant species** | 0-35 | 36-50 | 51-65 | 66-80 | 81-100 |
| **Trophic and reproductive function metrics** | | | | | |
| Number of invertivore species* | 8 or more | 6 or 7 | 4 or 5 | 2 or 3 | 0-1 |
| Number of piscivore species | 5 or more | 4 | 2 or 3 | 1 | 0 |
| Percent simple lithophils** | 61-100 | 46-60 | 31-45 | 16-30 | 0-15 |
| **Fish abundance and condition metrics** | | | | | |
| Number of fish per 100 meters* | 5 or more | | | | 0 |
| Percent DELT anomalies** | 0-1 | | 2 or 3 | | 4 or more |

\*Number of wetland species, number of invertivore species, and number of fish per 100 meters metrics do not include tolerant species

\*\*Round all percent metrics to the nearest 1 percent.


Table 20. Scoring criteria for the 10 metrics used to calculate the IBI for rivers (> 200 mi$^2$ drainage area) in the Upper Mississippi River Basin of Minnesota.

| Scoring Criteria | | | | | |
|---|---|---|---|---|---|
| Metric | 10 | 7 | 5 | 2 | 0 |
| **Species richness and composition metrics** | | | | | |
| Total number of species | 28 or more | 23-27 | 18-22 | 13-17 | 0-12 |
| Number of darter, sculpin, and madtom species | 4 or more | 3 | 2 | 1 | 0 |
| Number of intolerant species | 4 or more | 3 | 2 | 1 | 0 |
| Percent tolerant species** | 0-15 | 16-30 | 31-45 | 46-60 | 61-100 |
| **Trophic and reproductive function metrics** | | | | | |
| Number of invertivore species* | 14 or more | 11-13 | 8-10 | 5-7 | 0-4 |
| Percent omnivore species** | 0-10 | 11-20 | 21-30 | 31-40 | 41 or more |
| Number of piscivore species | 7 or more | 6 | 4 or 5 | 3 | 0-2 |
| Percent simple lithophils** | 81-100 | 61-80 | 41-60 | 21-40 | 0-20 |
| **Fish abundance and condition metrics** | | | | | |
| Number of fish per 100 meters | 1 | | 5 or more | | 0 |
| Percent DELT anomalies** | 0-1 | | 2 or 3 | | 4 or more |

\*Number of invertivore species and number of fish per 100 meters metrics do not include tolerant species.

\*\*Round all percent metrics to the nearest 1 percent.

Table 21. Scoring criteria for the nine metrics used to calculate the IBI for very small streams (< 20 mi$^2$ drainage area) in the St. Croix River Basin of Minnesota*.

| | Scoring Criteria | | | | |
|---|---|---|---|---|---|
| **Metric** | **10** | **7** | **5** | **2** | **0** |
| **Species richness and composition metrics** | | | | | |
| Total number of species | 10 or more | 8 or 9 | 6 or 7 | 4 or 5 | 0 - 3 |
| Number of headwater species** | 3 or more | | 1 or 2 | | 0 |
| Number of minnow species** | 5 or more | 4 | 2 or 3 | 1 | 0 |
| Percent tolerant species*** | 0-60 | 61-70 | 71 - 80 | 81-90 | 91-100 |
| Percent dominant two species*** | 0-52 | 53-64 | 65 - 76 | 77-88 | 89-100 |
| **Trophic composition and reproductive function metrics** | | | | | |
| Number of invertivore species** | 5 or more | 4 | 2 or 3 | 1 | 0 |
| Percent simple lithophils*** | 49-100 | 37-48 | 25-36 | 13-24 | 0-12 |
| **Fish abundance and condition metrics** | | | | | |
| Number of fish per 100 meters** | 11 or more | | | | 0-10 |
| Percent DELT anomalies*** | 0-1 | | 2 or 3 | | 4 or more |

*The sum of the nine metrics for headwater streams must be multiplied by 1.11 to obtain the final IBI score.
**Number of headwater species, number of minnow species, number of invertivore species, and number of fish per 100 meters metrics do not include tolerant species.
***Round all percent metrics to the nearest 1 percent.

Table 22. Scoring criteria for the nine metrics used to calculate the IBI for small streams (20 to 54 mi$^2$ drainage area) in the St. Croix River Basin of Minnesota*.

| | Scoring Criteria | | | | |
|---|---|---|---|---|---|
| **Metric** | **10** | **7** | **5** | **2** | **0** |
| **Species richness and composition metrics** | | | | | |
| Total number of species | 15 or more | 12-14 | 9-11 | 6-8 | 0-5 |
| Number of intolerant species | 4 or more | 3 | 2 | 1 | 0 |
| Number of minnow species** | 6 or more | 5 | 3 or 4 | 2 | 0 or 1 |
| Percent tolerant species*** | 0-40 | 41-55 | 56-70 | 71-85 | 86-100 |
| Percent dominant two species*** | 0-44 | 45-58 | 59-72 | 73-86 | 87-100 |
| **Trophic composition and reproductive function metrics** | | | | | |
| Number of benthic invertivore species | 4 or more | 3 | 2 | 1 | 0 |
| Percent simple lithophils3*** | 49-100 | 37-48 | 25-36 | 13-24 | 0-12 |
| **Fish abundance and condition metrics** | | | | | |
| Number of fish per 100 meters** | 11 or more | | | | 0–10 |
| Percent DELT anomalies*** | 0-1 | | 2 or 3 | | 4 or more |

*The sum of the 9 metrics for headwater streams must be multiplied by 1.11 to obtain the final IBI score.
**Number of minnow species, and number of fish per 100 meters metrics do not include tolerant species.
***Round all percent metrics to the nearest 1 percent.

Table 23. Scoring criteria for the ten metrics used to calculate the IBI for moderate size streams (55 to 270 mi$^2$ drainage area) in the St. Croix River Basin and rivers (>270 mi$^2$ drainage area) in the Northern Lakes and Forests ecoregion portion of the St. Croix River Basin in Minnesota.

| | Scoring Criteria | | | | |
|---|---|---|---|---|---|
| **Metric** | **10** | **7** | **5** | **2** | **0** |
| **Species richness and composition metrics** | | | | | |
| Total number of species | 23 or more | 20-22 | 17-19 | 14-16 | 0-13 |

| Metric | | | | | |
|---|---|---|---|---|---|
| Number of darter species | 5 or more | 4 | 3 | 2 | 0 or 1 |
| Number of intolerant species | 8 or more | 7 | 4-6 | 3 | 0-2 |
| Percent tolerant species* | 0-20 | 21-40 | 41-60 | 61-80 | 81-100 |
| **Trophic composition and reproductive function metrics** | | | | | |
| Number of benthic invertivore species | 9 or more | 7 or 8 | 5 or 6 | 3 or 4 | 0-2 |
| Number of omnivore species | 0 or 1 | 2 | 3 | 4 | 5 or more |
| Percent piscivore species* | 25-100 | 19-24 | 13-18 | 7-12 | 0-6 |
| Percent simple lithophils* | 61-100 | 46-60 | 31-45 | 16-30 | 0-15 |
| **Fish abundance and condition metrics** | | | | | |
| Number of fish per 100 meters** | 11 or more | | | | 0–10 |
| Percent DELT anomalies* | 0-1 | 2 or 3 | 4 or more | | |

*Round all percent metrics to the nearest 1 percent.
**Number of fish per 100 meters metrics does not include tolerant species.

Table 24. Scoring criteria for the ten metrics used to calculate the IBI for rivers (>270 mi$^2$ drainage area) in the North Central Hardwood Forests ecoregion portion of the St. Croix basin of Minnesota.

| | Scoring Criteria | | | | |
|---|---|---|---|---|---|
| **Metric** | **10** | **7** | **5** | **2** | **0** |
| **Species richness and composition metrics** | | | | | |
| Total number of species | 29 or more | 24-28 | 19-23 | 14-18 | 0-13 |
| Number of darter species | 5 or more | 4 | 3 | 2 | 0 or 1 |
| Number of intolerant species | 8 or more | 7 | 6 | 5 | 0-4 |
| Percent tolerant species* | 0-20 | 21-40 | 41-60 | 61-80 | 81-100 |
| **Trophic composition and reproductive function metrics** | | | | | |
| Number of benthic invertivore species | 11 or more | 9 or 10 | 7 or 8 | 5 or 6 | 0-4 |
| Number of omnivore species | 0 or 1 | 2 | 3 | 4 | 5 or more |
| Percent piscivore species* | 25-100 | 19-24 | 13-18 | 7-12 | 0-6 |
| Percent simple lithophils* | 61-100 | 46-60 | 31-45 | 16-30 | 0-15 |
| **Fish abundance and condition metrics** | | | | | |
| Number of fish per 100 meters** | 11 or more | | | | 0–10 |
| Percent DELT anomalies* | 0-1 | | 2 or 3 | | 4 or more |

*Round all percent metrics to the nearest 1 percent.
**Number of fish per 100 meters metric does not include tolerant species.

## PHYSICAL HABITAT ASSESSMENT

Habitat assessment is conducted using 13 transects evenly spaced along the length of the sampling station. At each transect five visual estimates or measurements are taken of habitat variables. Measurements are made of water depth, depth of fine sediments and water. Visual estimates are made of embeddedness of coarse substrates, and dominant substrate type. Five 0.3 × 0.3 meter quadrats are established across the transect from which percent of the quadrat containing algae and the percent of the quadrat containing emergent or submergent macrophytes are estimated. Cover and land use characteristics are estimated along the length of each transect including percent of the transect that contains various classes of fish cover, bank erosion, riparian land use, riparian buffer width, and canopy density. Channel characteristics are measured or estimated including channel condition, mean distance between bends, mean distance between riffles, total length of pools, runs, and riffles, and total number of pools, runs and riffles.

## REFERENCE SITE SELECTION

No related material.

## BIOLOGICAL CRITERIA

<u>Narrative</u>

The biological quality of any given surface water body shall be assessed by comparison to the biological integrity of a reference condition or conditions which best represents the most natural condition for that surface water body type within a geographic region. The biological quality shall be determined by reliable measures of indicative communities of fauna and flora.

<u>Numeric</u>

None

# IV.  STATE OF OHIO

Methodologies and regulations are detailed in a variety of references (Ohio EPA 1987; Ohio EPA 1987; Ohio EPA 1987; Rankin 1989; 1990; DeShon 1995).

## SAMPLING REACH CONSIDERATIONS

Selection of fish community sampling sites used by Ohio EPA are based upon the following factors.  1) Location of point source discharges.  2) Stream use designation evaluation issues.  3) Location of physical habitat features.  4) Location of non-point sources of pollution.  5) Variations in habitat.  Sample sites should contain typical and representative habitat to that of the segment under study.  Sites downstream of pollution sources are usually supplemented with data from control sites upstream of the pollution source.  If no control sites are available upstream of the study area, then control sites may be chosen from adjacent streams with similar physical characteristics.  Ecoregional reference sites may also be used.

Sample stations have a length of 150 to 200 meters for wadeable and headwater stream sites, and 500 meters for non-wadeable stream sites.

## MACROINVERTEBRATE COMMUNITY ASSESSMENT

### FIELD METHODS AND INDEX PERIOD

Quantitative Sampling

> The primary sampling equipment used by Ohio EPA is the modified Hester-Dendy multi-plate artificial substrate sampler.  The surface area of the sampler totals 145.6 square inches.  Hester-Dendy samplers are placed in runs whenever this habitat type is available.  Colonization time is six weeks.  Three or five samplers are placed per sample site.  Surber square foot samplers are also used.  Quantitative samples from deep water are taken with Ekman, Peterson, or Ponar samplers.  Five samples are taken with the respective deepwater gear.  Ohio EPA samples macroinvertebrates from June 15 to September 30. Sampling that occurs outside this window needs to be scrutinized since reference values are calibrated to sites sampled within the indexing period.

Qualitative Sampling

> D-frame kick nets, Ekman, Peterson, and Ponar are used for qualitative sampling.  Sampling takes place near the artificial substrate quantitative sampling devices.  All available habitats are sampled until no new taxa appear in subsequent samples.  When qualitative samples are taken without quantitative sampling the sampling effort is increased substantially.  Qualitative only samples are taken for a minimum of 30 minutes and may continue longer than 30 minutes if further sampling generates new taxa.

Field Preservation

> 70% ETOH

### LABORATORY METHODS

Level of Taxonomic Identification

> Lowest possible level.

Laboratory Preservation

70% ETOH
10% KOH used for clearing Chironomidae species.

CMCP 9/9AF or Euparal mounting mediums used for mounting Chironomidae species.

Subsampling

Quantitative samples are either composited or used as replicates, depending on study objectives, *e.g.*, routine monitoring or litigation purposes. Organisms and material are sieved through a standard number 30 then standard number 40 standard testing sieves. Folsom sample splitters are used for all subsampling after sorting and removing large/rare taxa. Very large sampled may be split before sorting. After sorting an entire sample to the family level, families that have unmanageable numbers of individuals may be subsampled. At least 250 organisms must be contained in a subsample, including 50-100 midges, 70 caddisflies, and 70 mayflies.

DATA ANALYSIS AND INTERPRETATION

Ohio EPA uses several indices and coefficients for describing in the analysis of benthic macroinvertebrate communities. The community measures are then related to environmental variables measured or estimated during the course of a site visit. Community composition metrics include Shannon's diversity index, Lloyd and Ghelardi's expression of the Equitability Index, Van Horn's Community Similarity index. Spearman's Rank Correlation Coefficient is used to describe the relationship between measured biological, chemical, or physical data. Coefficient of Variation is calculated for replicate samples.

Ohio EPA's Invertebrate Community Index (ICI) is the principal measure of overall community condition used by its staff. Ten metrics are scored and assigned points according to one of four numeric scoring categories; 6 points if a metric has a value comparable to that of an exceptional stream community; 4 points for values typical of a good stream community; 2 points for values deviating slightly from values typical of a good stream community; and 0 points for values deviating strongly from values typical of a good stream community.

Table 25. Ohio EPA's Invertebrate Community Index metrics and scoring criteria.

| | Scoring Criteria | | | |
|---|---|---|---|---|
| Metric | 0 | 2 | 4 | 6 |
| Total Number of Taxa | | Varies With Drainage Area | | |
| Total Number of Mayfly Taxa | | Varies With Drainage Area | | |
| Total Number of Caddisfly Taxa | | Varies With Drainage Area | | |
| Total Number of Dipteran Taxa | | Varies With Drainage Area | | |
| Percent Mayflies | 0 | 0-10 | 10-25 | >25 |
| Percent Caddisflies | | Varies With Drainage Area | | |
| Percent Tribe Tanytarsini Midges | 0 | 0-10 | 10-25 | >25 |
| Percent Other Dipterans and Non-insects | | Varies With Drainage Area | | |
| Percent Tolerant Organisms | | Varies With Drainage Area | | |
| Total Number of Qualitative EPT Taxa | | Varies With Drainage Area | | |

**FISH COMMUNITY ASSESSMENT**

FIELD METHODS

Pulsed DC electrofishing is the primary fish collection method used by Ohio EPA, however eleven methods are described as valid for use in their biological assessment program. Non-wadeable streams are sampled using a boat-mounted electrofishing unit. Backpack electrofishing units, Sportyak-generator units

(*e.g.,* towed barge unit), longline generator units, gill nets, Fyke nets, hoop nets, trap nets, and seines are also used.

Sample reaches for boat electrofishing are at least 0.5 kilometer and fished in a downstream direction. A single pass is made; subsequent passes are made in five to six week intervals.
Fish samples are weighed collectively, by species, or individually.

Field Preservation

> 10% formalin solution buffered with one teaspoon of borax per ½ gallon.

LABORATORY METHODS

Laboratory Preservation

> Fish are fixed in a buffered 10% formalin solution for 2 to 3 weeks. Formalin is drained from the fish for ½ hour before being placed in 35% ETOH for another 2 to 3 weeks. Fish are then transferred to 50% ETOH for another 2 to 3 weeks before being placed in 70% ETOH for permanent storage.

DATA ANALYSIS AND INTERPRETATION

Relative abundance data is generated in terms of numbers per species per unit distance or weight per species per unit distance for active sampling gear, numbers per unit time for passive gear. Measures of community composition and characteristics include total number of species per sample, cumulative number of species per sampling location, Shannon' Diversity based on numbers of individuals and weight of individuals, modified Index of Well-Being, and the Index of Biological Integrity.

**PHYSICAL HABITAT ASSESSMENT**

Ohio EPA uses a habitat assessment called the Qualitative Habitat Evaluation Index (QHEI) (Rankin 1989). This index provides a measure of macrohabitat quality factors that affect the quality of habitat available for fish communities and also those habitat characteristics that generally important to groups of aquatic invertebrates.

The QHEI consist of 18 parameters contained in six broad categories. The scoring range for QHEI is from 0 to 100. Table 26 shows the parameters their respective scoring ranges.

Table 26. Ohio EPA's QHEI main parameter categories, parameters score ranges, and total potential points per category.

| Parameter Category | Subcategory | Potential Points/Subcategory | Parameter Score Range |
|---|---|---|---|
| **Substrate** | | | **20** |
| | Type | 0 - 20 | |
| | Quality | -5 - 3 | |
| **Instream Cover** | | | **20** |
| | Type | 0 - 9 | |
| | Amount | 1 - 11 | |
| **Channel Quality** | | | **20** |
| | Sinuosity | 1 - 4 | |
| | Development | 1 - 7 | |
| | Channelization | 1 - 6 | |
| | Stability | 1 - 3 | |
| **Riparian/Erosion** | | | **10** |

| | | | |
|---|---|---|---|
| | Width | 0 - 4 | |
| | Floodplain Quality | 0 - 3 | |
| | Bank Erosion | 1 - 3 | |
| **Pool/Riffle** | | | **20** |
| | Maximum Depth | 0 - 6 | |
| | Current Available | -2 - 4 | |
| | Pool Morphology | 0 - 2 | |
| | Riffle/Run Depth | 0 - 4 | |
| | Riffle Substrate Stability | 0 - 2 | |
| | Riffle Embeddedness | -1 - 2 | |
| **Gradient** | | 0 - 10 | **10** |

## REFERENCE SITE SELECTION

Identification

Map detailing human population density, number and size of point discharges, and current and past land use patterns are used to determine least-impacted watersheds within an ecoregion. Candidate sites within these watersheds are examined aerially for desirable and representative features as well as examined on the ground. Ground examination includes biological and physical habitat assessment.

## BIOLOGICAL CRITERIA

Narrative

Biological criteria presented in table 7-17 (see numeric criteria section) to this rule provide a direct measure of the attainment of the warm water habitat, exceptional warm water habitat and modified warm water habitat aquatic life uses. Biological criteria and the exceptions to chemical-specific or whole-effluent criteria allowed by this paragraph do not apply to any other use designations.
(a) Demonstrated attainment of the applicable biological criteria in a water body will take precedence over the application of chemical-specific or whole-effluent criteria associated with these uses when the director, upon considering appropriately detailed chemical, physical and biological data, finds that one or more chemical-specific or whole-effluent criteria are inappropriate. In such cases the options that exist include:
(a)(i) The director may develop, or a discharger may provide for the director's approval, a justification for a site-specific water quality criterion according to methods described in "Water Quality Standards handbook, 1983, U.S. EPA Office of Water";
(a)(ii) The director may proceed with establishing water quality based effluent limits consistent with attainment of the designated use.
(b) Demonstrated nonattainment of the applicable biological criteria in a water body with concomitant evidence that the associated chemical-specific criteria and whole-effluent criteria are met will cause the director to seek and establish, if possible, the cause of the nonattainment of the designated use. The director shall evaluate the existing designated use and, where not attainable, propose to change the designated use. If the designated use is deemed attainable, the director shall, whenever possible and reasonable, implement regulatory controls or make other recommendations regarding water resource management to restore the designated use.

Definitions-

"Warmwater"- these are waters capable of supporting and maintaining a balanced, integrated, adaptive community of warmwater aquatic organisms having a species composition, diversity, and functional organization comparable to the twenty-fifth percentile of the identified reference sites within each of the following ecoregions: the interior plateau ecoregion, the Erie/Ontario lake plains ecoregion, the western Allegheny plateau ecoregion and eastern corn belt plains ecoregion. For the Huron/Erie lake plains ecoregion, the comparable species composition, diversity and functional organization are based upon the

ninetieth percentile of all sites within the ecoregion. For all ecoregions, the attributes of species composition, diversity and functional organization will be measured using the index of biotic integrity, the modified index of well-being and the invertebrate community index as defined in "Biological Criteria for the Protection of Aquatic Life: volume II, Users Manual for Biological field Assessment of Ohio Surface Waters," ... Attainment of this use designation is based on the criteria in table 7-17 to this rule. A temporary variance to the criteria associated with this use designation may be granted as described in paragraph (G) of rule 3745-1-01 of the Administrative Code.

"Exceptional Warmwater" - these are waters capable of supporting and maintaining an exceptional or unusual community of warmwater aquatic organisms having a species composition, diversity, and functional organization comparable to the seventy-fifth percentile of the identified reference sites on a statewide basis. The attributes of species composition, diversity and functional organization will be measured using the index of biotic integrity, the modified index of well-being and the invertebrate community index as defined in "Biological Criteria for the Protection of Aquatic Life: volume II, Users Manual for Biological field Assessment of Ohio Surface Waters,". In addition to those stream segments designated in rules 3745-1-08 to 3745-1-32 of the Administrative Code, all lakes and reservoirs, except upground storage reservoirs, are designated exceptional warmwater habitats. Attainment of this use designation (except for lakes and reservoirs) is based on the criteria in table 7-17 to this rule. A temporary variance to the criteria associated with this use designation may be granted as described in paragraph (G) of rule 3745-1-01 of the Administrative Code.

"Modified Warmwater" - these are waters that have been the subject of a use attainability analysis and have been found to be incapable of supporting and maintaining a balanced, integrated, adaptive community of warmwater organisms due to irretrievable modifications of the physical habitat. Such modifications are of a long-lasting duration (*i.e.*, twenty years or longer) and may include the following examples: extensive stream channel modification activities permitted under sections 401 and 404 of the act or Chapter 6131 of the Revised Code, extensive sedimentation resulting from abandoned mine land runoff, and extensive permanent impoundment of free-flowing water bodies. The attributes of species composition The attributes of species composition, diversity and functional organization will be measured using the index of biotic integrity, the modified index of well-being and the invertebrate community index as defined in "Biological Criteria for the Protection of Aquatic Life: volume II, Users Manual for Biological field Assessment of Ohio Surface Waters,". Attainment of this use designation is based on the criteria in table 7-17 to this rule. Each water body designated modified warmwater habitat will be listed in the appropriate use designation rule (rules 3745-1-08 to 3745-1-32 of the Administrative Code) and will be identified by ecoregion and type of physical habitat modification as listed in table 7-17 to this rule. The modified warmwater habitat designation can be applied only to those waters that do not attain the warmwater habitat designation that do not attain the warmwater habitat biological criteria in table 7-17 to this rule because of irretrievable modifications of the physical habitat. All stream segments designated modified warmwater habitat will be reviewed on a triennial basis (or sooner) to determine whether the use designation should be changed. A temporary variance to the criteria associated with this use designation may be granted as described. in paragraph (G) of rule 3745-1-01 of the Administrative Code.

Numeric

Table 27. State of Ohio Numeric Biological Criteria for waters of the state.

| Ohio Numeric Biocriteria | | | | | |
|---|---|---|---|---|---|
| Biological criteria for Warm water, Exceptional Warm water, and Modified Warm water Habitats. Description and derivation of indices and ecoregions are contained in "Biological Criteria for the Protection of Aquatic Life: Volume II. Users Manual for Biological Field Assessment of Ohio Surface Waters" cited in paragraph (B) of Rule 3745-1-03 of the Administrative Code. These criteria do not apply to the Ohio River, lakes or Lake Erie river mouths. | | | | | |
| **Modified Warm Water Habitat** | | | | | |
| **Index Sampling Site Ecoregion**[1] | **Channel Modification** | **Mine Affected** | **Impounded** | **Warm water** | **Exceptional Warm water** |

| | | | | Habitat | Habitat |
|---|---|---|---|---|---|
| **I. Index of Biotic Integrity (Fish)** | | | | | |
| **A. Wading Sites[2]** | | | | | |
| HELP | 22 | -- | -- | 32 | 50 |
| IP | 24 | -- | -- | 40 | 50 |
| EOLP | 24 | -- | -- | 38 | 50 |
| WAP | 24 | 24 | -- | 44 | 50 |
| ECBP | 24 | -- | -- | 40 | 50 |
| **B. Boat Sites[2]** | | | | | |
| HELP | 20 | -- | 22 | 34 | 48 |
| IP | 24 | -- | 30 | 38 | 48 |
| EOLP | 24 | -- | 30 | 40 | 48 |
| WAP | 24 | 24 | 30 | 40 | 48 |
| ECBP | 24 | -- | 30 | 42 | 48 |
| **C. Headwater Sites[3]** | | | | | |
| HELP | 20 | 20 | -- | 28 | 50 |
| IP | 24 | 24 | -- | 40 | 50 |
| EOLP | 24 | 24 | -- | 40 | 50 |
| WAP | 24 | 24 | 24 | 44 | 50 |
| ECBP | 24 | 24 | -- | 40 | 50 |
| **II. Modified Index of Well-Being (Fish)[4]** | | | | | |
| **A. Wading Sites[2]** | | | | | |
| HELP | 5.6 | -- | -- | 7.3 | 9.4 |
| IP | 6.2 | -- | -- | 8.1 | 9.4 |
| EOLP | 6.2 | -- | -- | 7.9 | 9.4 |
| WAP | 6.2 | 5.5 | -- | 8.4 | 9.4 |
| ECBP | 6.2 | -- | -- | 8.3 | 9.4 |
| **B. Boat Sites[2]** | | | | | |
| HELP | 5.7 | -- | 5.7 | 8.6 | 9.6 |
| IP | 5.8 | -- | 6.6 | 8.7 | 9.6 |
| EOLP | 5.8 | -- | 6.6 | 8.7 | 9.6 |
| WAP | 5.8 | 5.4 | 6.6 | 8.6 | 9.6 |
| ECBP | 5.8 | -- | 6.6 | 8.5 | 9.6 |
| **III. Invertebrate Community Index (Macroinvertebrates)** | | | | | |
| **A. Artificial Substrate Samplers[2]** | | | | | |
| HELP | 22 | -- | -- | 34 | 46 |
| IP | 22 | -- | -- | 30 | 46 |
| EOLP | 22 | -- | -- | 34 | 46 |
| WAP | 22 | 30 | -- | 36 | 46 |
| ECBP | 22 | -- | -- | 36 | 46 |

[1] HELP = Huron/Erie Lake Plain Ecoregion. IP = Interior Plateau Ecoregion. EOLP = Erie/Ontario Lake Plain Ecoregion. WAP = Western Allegheny Plateau Ecoregion. ECPB = Eastern Corn Belt Plains Ecoregion

[2] Sampling methods descriptions are found in the "Manual of Ohio EPA Surveillance Methods and Quality Assurance Practices," cited in paragraph (B) of Rule 3745-1-03 of the Administrative Code.

[3] Modification of the IBI that applies to sites with drainage areas less than 20 square miles.

[4] Does not apply to sites with drainage areas less than 20 square miles.

(Effective February 14, 1978; April 4, 1985; August 19, 1985; April 30, 1987; May 1, 1990)

# COMPOSITE SUMMARY OF PROGRAMMATIC ELEMENTS OF STATE AND NATIONAL ENTITIES

# I. SUMMARY OF BIOLOGICAL MONITORING COMPONENTS, SAMPLING PERIODS, AND REFERENCE CONDITIONS.

We have restricted our review of biological monitoring programs and methods to those geo-political entities that most likely manage similar ecosystems and have similar management goals. Most methods included in this review are associated with state programs and are often based on methods developed by and used in many federal environmental programs.  We believe that a close examination of existing programs and their methods can be used to select or modify biological monitoring design components and methods most suited to the long-term monitoring of aquatic resources located on and influencing the environmental quality of USEPA Region 7.  The following compiled information and summary statements suggest that certain "core" approaches and methods are common to many environmental monitoring programs.  The commonality of many methods and monitoring components indicates these elements are robust, have broad applicability and are easily adopted (or modified) for use within the region.  These monitoring methods have been developed and time tested by managers of similar ecosystems with similar goals.  Using these methods would have two major benefits: 1) it would facilitate outside comparisons by reducing differences in information based on varying methods, and 2) it would expand the ability to assess the condition of aquatic resources through time and space by comparison with regional data from similar resource populations (e.g. reference lakes and streams, healthy stream segments).

Eleven state and national biological monitoring programs were examined.  All programs included habitat and macroinvertebrate monitoring components that were monitored during normal or base flow conditions, most often occurring in the growing season.  Generally, state and federal programs try to sample macroinvertebrates communities between spring and late fall.  Nearly all programs used a reference condition/site approach to evaluate relative conditions associated with monitoring sites.  Most programs used ranking systems based on objective data to classify biological conditions (e.g. excellent, moderately impaired, etc.).  The following specific commonalities were observed in our evaluations of the reviewed programs:

1.  All (11 of 11) reviewed protocols recommend physical habitat assessments and monitor macroinvertebrate communities.  Almost all (10 of 11) recommend monitoring of fish communities, and few (2 of 11) recommend monitoring of algal communities (Table 28).

2.  All (11 of 11) specify sampling at base flow conditions, and most (8 of 11) sampling periods fall within the growing season for macroinvertebrates (Table 29).

3.  Most (8 of 11) protocols designate some method for determination of reference sites or conditions.  For those protocols that designate methods of determination, the two most common are best professional judgment (4 of 8) and least disturbance (4 of 8) (Table 30).

Table 28. Primary programmatic elements of biological assessment programs of state and national regulatory and management entities.

| Monitoring Entity | Invertebrates | Fish | Algae | Habitat |
|---|---|---|---|---|
| Iowa | X | X | | X |
| Kansas | X | X | | X |
| Missouri | X | | | X |
| Nebraska | X | X | | X |
| USEPA | X | X | X | X |
| USGS | X | X | X | X |
| USFS | X | X | | X |
| Arkansas | X | X | | X |
| Indiana | X | X | | X |
| Minnesota | X | X | | X |
| Ohio | X | X | | X |

The programmatic elements compiled in Table A are primarily elements related to the monitoring and assessment of running water environments. Few state and federal programs have established monitoring guidelines for wetlands. However, the monitoring and assessment of lakes and reservoirs is common and relies mainly on water chemistry data and measurements of planktonic chlorophyll (an indicator of algal biomass) and the areal extent vascular plant communities. Detailed habitat evaluations for lakes and reservoirs are seldom part of programmatic efforts, but general morphology data (e.g. lake volume, mean depth, water residence time) is determined for many of the larger lakes and reservoirs within environmental programs. Occasionally, fish and invertebrate monitoring is designed into monitoring programs, but the collection and evaluation of such data tends to be sporadic within state and federal programs in the mid-continent area.

Table 29. Index periods and sampling regimes of biological assessment programs of state and national regulatory and management entities.

| Monitoring Entity | Index Period(s) | Sampling Regime | |
|---|---|---|---|
| | | Visits/ Site/Year | Samples/Site |
| Iowa | Jul 15 - Oct 15 | 1 | 1 or 3 |
| Kansas[1] | Spring, Summer, Fall | 1 in rotating seasons | 2 |
| Missouri | Sep 15 - Oct 15 | 1 | 1 composite |
| Nebraska[2] | May - Sep# | 1 | 1 |
| USEPA | No guideline | 1 | 1 composite |
| USGS[2] | No guideline | 1 | 1 |
| USFS | Year round | 4 (1 per season) | 3 |
| Arkansas | Apr - Jun Sep - Oct | 1 | 1 composite |
| Indiana | Jul - Oct | No more than 1 every other yr | No guideline |
| Minnesota[3] | Jun - Jul | 1 | 1 composite |
| Ohio | Jun 15 - Sep 30 | 1 | 3 - 5 |

[1] Spring one year, Summer the second year, Fall the third, then repeat.
[2] No specific guideline; determined from associated data.
[3] Earlier sampling allowed based on temperature.

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Table 30. Approaches used to define and identify reference conditions and sites employed in biological assessment programs of state and national regulatory and management entities.

| Monitoring Entity | Determination of Reference Sites | | | | | | |
|---|---|---|---|---|---|---|---|
| | Historical Conditions | Paired Watersheds | Best Professional Judgment | Objective Data | Least Disturbed | Statistical Distribution | No Guidance/ Under Development |
| Iowa | | | | | | X | |
| Kansas | | | | | X | | |
| Missouri | | | | | X | | |
| Nebraska | | | | | X | | |
| USEPA | | X | X | | | | |
| USGS | X | | X | | | | |
| USFS | | | | | | | X |
| Arkansas | | | | | | | X |
| Indiana | | | X | | | | |
| Minnesota | | | | | | | X |
| Ohio | X | | X | | X | | |

Several categories listed above represent general concepts that incorporate the use of diverse variables and factors, depending upon the protocol. In addition, while different groups use similar factors or variables, they may or may not be measured quantitatively. Nearly all approaches can involve subjective evaluations of factors and their "weighting value" in selecting reference sites. "Historical conditions" refers to the use of historic data in determining typically pre-settlement or un-disturbed conditions. However, if major land use changes have occurred (e.g. urban growth areas), historic data can be data that is only several decades old. Often, historic data suffers from a lack of data quality information and may not have the level of resolution necessary to address regional or local issues. "Paired watersheds" refers to the use of watersheds of similar type and size that have known differences in potential stressors. This approach works well if the study objectives are limited in spatial area and address issues that are more " site specific" in nature. "Best professional judgment (BPJ)" represents an approach that relies upon the subjective knowledge and experience of involved personnel to determine whether or not an aquatic resource is free enough from impairments to be considered a reference system or site. Often the type and amount of quantitative or qualitative information varies considerably among people and organizations; thus, BPJ suffers from information inconsistencies and inherent evaluation biases related to individual and collective educational and work experiences. It is suggested that BPJ is often a beginning point in the selection process, and that BPJ results be further evaluated as a more formal method is adopted and data needs are addressed. The "Least disturbed" approach to identifying reference conditions and sites is based on the identification and evaluation of stressor and receptors in aquatic ecosystems of interest and their watersheds. Typically, factors relating to water quality, in-stream and near-habitat conditions, and watershed or landscape variables and potential stressors are used to evaluate reference sites, and biological variables are used to validate and test these evaluations. Minimally impacted areas are considered reference sites. When dealing with large populations of similarly classified aquatic systems (e.g. wadeable streams) a "Statistical distribution" approach is used when reference conditions and sites are either unknown or absent. The

statistical distribution of factors or condition values is then used to determine a relative ranking of conditions and/or sites, based on some assessed statistical property(s) such as an upper or lower quartile. The final category (No guidance/under development) was used to identify entities that have not explicitly described the reference site determination process.

**II. SUMMARY OF MACROINVERTEBRATE COMMUNTIY MONITORING PROTOCOLS.**

Further evaluation of the macroinvertebrate programs and protocols examined in this review revealed a number of common factors.  Most groups recommend use of at least semi-quantitative collection methods, often sampling with either a D-framed net over multiple habitats, or artificial substrate samplers for a prescribed time period.  Monitoring groups working in stream systems with well-defined and reoccurring rifle areas in stream segments of interest have recommended the use of a quantitative riffle sampler such as a Hess or Surber sampler.  Additional non-riffle areas required the use of different sampling gear.

The majority of approaches used replicated samples (typically 3 to 5 samples) in assessing site and system conditions.  Within the monitoring programs evaluated, macroinvertebrate samples were most often systematically subsampled to reduce data processing time and expenses.  The raw data was then used to generate a number of evaluation metrics. All but one of the monitoring programs used these metrics to create a multimetric index and index scoring scenario that was used in interpreting temporal and spatial change in the macroinvertebrate community within and among sites (and streams).  Eighty different metrics were used in at least one or more of the 11 programs surveyed, while only 16 different metrics were noted to be included in three or more of the programs.  Only 10 metrics were found to be common to four or more of the programs using macroinvertebrates for monitoring purposes.  By far the most frequently used metrics were taxa richness, number of EPT taxa, percent dominant taxa and scores from the Hilsenhoff Biotic Index.  The following generalities were observed among the surveyed entities that used macroinvertebrates in a biological monitoring program:

1. Most protocols (9 of 11) require semi-quantitative, multi-habitat sampling, and some (4 of 11) require qualitative, multi-habitat sampling.  Relatively few protocols require single habitat sampling, either qualitatively (1 of 11) or semi-quantitatively (2 of 11) (Table 31).

2. Two types of sampling equipment were used in more than 33% of protocols: D-frame kick nets (6 of 11) and Artificial Substrate samplers (4 of 11) (Table 32, Table 33).

3. A majority of protocols recommend replicate samples (6 of 11), subsampling (7 of 11), and multimetric indices (10 of 11) (Table 34).

4. Ten primary metrics were used in at least 25% (i.e. 4 or more) of the protocols:
   | | |
   |---|---|
   | % Chironomidae | Total Number of Taxa |
   | % Ephemeroptera | Number of Dipteran Taxa |
   | % Trichoptera | Number of Ephemeropteran Taxa |
   | % Dominant taxon | Number of Trichopteran Taxa |
   | Hilsenhoff Biotic Index | Number of EPT Taxa |

Table 31. Comparison of qualitative and semi-quantitative macroinvertebrate protocols used in biological assessment programs of state and national regulatory and management entities.

| Monitoring Entity | Qualitative | | Semi-quantitative | |
|---|---|---|---|---|
| | Single Habitat | Multi-habitat | Single Habitat | Multi-habitat |
| Iowa | | X | | X |
| Kansas | | | | X |
| Missouri | | | | X |
| Nebraska | | X | | X |
| USEPA | | | | X |
| USGS | | X | | X |
| USFS | | | | X |
| Arkansas | | | X | X |
| Indiana | | | | X |
| Minnesota | X | | | |
| Ohio | | X | X | |

Table 32. Invertebrate sampling equipment used in qualitative sampling efforts in biological assessment programs of state and national regulatory and management entities.

| Monitoring Entity | Sampling Equipment | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | D-net | Eckman | Ponar | Peterson | Surber | Artificial Substrate | Hand Picking | Modified Hess |
| Iowa | | | | | | X | X | |
| Kansas | X | | | | | | | |
| Missouri | | | | | | | | |
| Nebraska | X | | | | | | X | |
| USEPA | | | | | | | | |
| USGS | X | X | X | | | | X | |
| USFS | | | | | | | | |
| Arkansas | | | | | | | | |
| Indiana | | | | | | | | |
| Minnesota | X | | | | | | | |
| Ohio | X | X | X | X | | | | |

Table 33. Invertebrate sampling equipment used in quantitative sampling efforts in biological assessment programs of state and national regulatory and management entities.

| Monitoring Entity | Sampling Equipment | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | D-net | Eckman | Ponar | Peterson | Surber | Artificial Substrate | Hand Picking | Modified Hess | Brown |
| Iowa | | | | | | X | | X | |
| Kansas | X | | | | | | | | |
| Missouri | X | | | | | | X | | |
| Nebraska | X | | X | X | X | X | | | |
| USEPA | X | | | | | | | | |
| USGS | X | X | X | | | | | | |
| USFS | | | | | X | X | | X | |
| Arkansas | X | | | | | | | | |
| Indiana | | | | | | | | | |
| Minnesota | | | | | | | | | |
| Ohio | | X | X | X | | X | | | |

Table 34. Methods used for data analysis and interpretation by state and national entities for the evaluation of macroinvertebrate data.

| Monitoring Entity | Replicate Samples | Sub-sampling | Multi-metric Indices | Multi-variate Indices | Hypothesis Testing | Level of Taxonomic Identification |
|---|---|---|---|---|---|---|
| Iowa | X | X | X | | | lowest practical |
| Kansas | X | | X | | | lowest practical |
| Missouri | X | X | X | | | lowest practical |
| Nebraska | | X | X | | | lowest practical |
| USEPA | | X | X | X | | lowest practical |
| USGS | | | | | | lowest practical |
| USFS | X | | X | | | genus or species |
| Arkansas | | X | X | | | lowest practical |
| Indiana | | X | X | | | family |
| Minnesota | X | | X | | | genus |
| Ohio | X | X | X | | | lowest practical |

### III. SUMMARY OF FISH COMMUNITY MONITORING PROTOCOLS .

Ten of the 11 evaluated biological monitoring programs (all but Missouri) recommended the biological monitoring of fish communities, but only 9 of these 10 programs (all but Indiana) provided the specific protocols used in their fish monitoring efforts (Indiana had provided no guidance at the time of this report). Most programs identified the minimum length of the stream reach to be sampled, as well as the types of habitats included in their sampling effort.  For those programs that listed specific collection methods, some form of seining and electrofishing were the primary sampling methods.  Most programs developed and used a number of fish metrics, and those metrics were typically combined into a multimetric index for purposes of evaluation.  The following generalities were observed among the ten protocols that required biological monitoring of fish:

1. More than half (7 of 10) of the protocols recommended a defined sampling reach length; of those protocols that recommended defined reach lengths, most (5 of 7) recommended lengths with upper and lower limits.  Half of the protocols recommended the use of block nets to restrict immigration and emigration of fish from the sampling reach.  More than half of the programs recommended multi-habitat sampling for fish (Table 35).

2. The two most common types of equipment recommended for fish sampling were backpack electrofishing equipment (9 of 10) and seines (6 of 10) (Table 36).

3. Multimetric indices were recommended in 70% of protocols that monitor fish (7 of 10) (Table 37).

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Table 35. Elements of field fish sampling protocols used in biological assessment programs of state and national regulatory and management entities.

| Monitoring Entity | Sampling Reach Length | Block Nets Used | Single Habitat Sampling | Multi-Habitat Sampling | Avoid Areas of Human Impact |
|---|---|---|---|---|---|
| Iowa | 150-500 m | | | | |
| Kansas | 150-300 m | X | | X | X |
| Missouri [1] | 20 times the mean width | | | | |
| Nebraska | | X | | X | |
| USEPA | 150-300 m | X | | X | X |
| USGS | 150-300 m | X | | X | |
| USFS | | X | | | |
| Arkansas | | | | | |
| Indiana | | | | | |
| Minnesota | 35 times the mean width | | | X | |
| Ohio | 500 m | | | X | |

[1] Missouri does not require biological monitoring of fish. This sampling reach length is for physical habitat and macroinvertebrate monitoring purposes.

Table 36. Fish sampling equipment used in biological assessment programs of state and national regulatory and management entities.

| Monitoring Entity | Seine | Backpack Electrofishing | Tote Barge Electrofishing | Boat Mounted Electrofishing | Passive Sampling Gear |
|---|---|---|---|---|---|
| Iowa | | X | X | | |
| Kansas | X | X | | | |
| Missouri [1] | | | | | |
| Nebraska | X | X | X | X | X |
| USEPA | X | X | | | |
| USGS | X | X | | X | |
| USFS | X | X | | | |
| Arkansas | X | X | | X | X |
| Indiana | | | | | |
| Minnesota | | X | X | | |
| Ohio | | X | | X | X |

[1] Missouri does not require biological monitoring of fish.

Table 37. Methods used for data analysis and interpretation by state and national entities for the evaluation of fish data.

| Monitoring Entity | Replicate Samples | Sub-sampling | Multimetric Indices | Multivariate Indices | Hypothesis Testing |
|---|---|---|---|---|---|
| Iowa | | | X | | |
| Kansas | | | X | | |
| Missouri [1] | | | | | |
| Nebraska | | | X | | |
| USEPA | | | X | X | |
| USGS | | | | | |
| USFS | | | | | |
| Arkansas | | | | | |
| Indiana | | | X | | |
| Minnesota | | | X | | |
| Ohio | | | X | | |

[1]  Missouri does not require biological monitoring of fish.

**IV. SUMMARY OF ALGAL COMMUNITY MONITORING PROTOCOLS.**

None of the evaluated state programs, either within or outside USEPA Region 7, used algae in a biological monitoring program.  Two national biological monitoring protocols recommended biological monitoring of algal communities.  Often, chlorophyll concentrations were identified as a measurement variable.  These two programs recommended periphyton (i.e. attached algae) monitoring, using both qualitative and quantitative methods (Table 38).  Subsampling was also used in both programs to generate community level variables (Table 39).

Table 38. Algal communities sampled and protocol type used in biological assessment programs of state and national regulatory and management entities.

| Monitoring Entity | Periphyton | Phytoplankton | Filamentous Algal Mats | Qualitative | Quantitative |
|---|---|---|---|---|---|
| Iowa | | | | | |
| Kansas | | | | | |
| Missouri | | | | | |
| Nebraska | | | | | |
| USEPA | X | | | X | X |
| USGS | X | X | X | X | X |
| USFS | | | | | |
| Arkansas | | | | | |
| Indiana | | | | | |
| Minnesota | | | | | |
| Ohio | | | | | |

Table 39. Methods used for data analysis and interpretation by state and national entities for the evaluation of algae data.

| Monitoring Entity | Replicate Samples | Sub-sampling | Multi-metric Indices | Multi-variate Indices | Hypothesis Testing | Level of Taxonomic Identification |
|---|---|---|---|---|---|---|
| Iowa | | | | | | |
| Kansas | | | | | | |
| Missouri | | | | | | |
| Nebraska | | | | | | lowest practical |
| USEPA | | X | X | X | | |
| USGS | | X | | | | species |
| USFS | | | | | | |
| Arkansas | | | | | | |
| Indiana | | | | | | |
| Minnesota | | | | | | |
| Ohio | | | | | | |

## V. SUMMARY OF PHYSICAL HABITAT ASSESSMENT PROTOCOLS.

All 11 of the evaluated state and national biological monitoring protocols recommended physical habitat assessment in conjunction with biological monitoring.  Among these, the following generalities were observed:

All (11 of 11) recommended visual based assessment methods.  Most (9 of 11) additionally recommended quantitative methods, and many (8 of 11) also recommended habitat development indices (Table 40).

Table 40. Habitat assessment methods used in biological assessment programs of state and national regulatory and management entities.

| Monitoring Entity | Visual Based | Quantitative | Habitat Development Indices |
|---|---|---|---|
| Iowa | X | | |
| Kansas | X | X | X |
| Missouri | X | X | X |
| Nebraska | X | X | X |
| USEPA | X | X | X |
| USGS | X | X | X |
| USFS | X | X | |
| Arkansas | X | | X |
| Indiana | X | X | X |
| Minnesota | X | X | |
| Ohio | X | X | X |

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# APPENDIX I. REFERENCES

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## APPENDIX II. GLOSSARY

**Acclimation -** response by an animal that enables it to tolerate a change in a single factor (e.g. temperature) in its environment.

**Adaptation -** adjustments made by animals in respect of their environments. The adjustments may occur by natural selection, as individuals with favorable genetically acquired traits breed more prolifically than those lacking these traits (genotypic adaptation), or they may involve non-genetic changes in individuals, such as physiological modification (e.g. acclimatization) or behavioral changes (phenotypic adaptation).

**Aquatic Assemblage -** an organism group of interacting populations in a given waterbody, for example, fish assemblage or a benthic macroinvertebrate assemblage.

**Aquatic Biota -** collective term describing the organisms living in or depending on the aquatic environment.

**Aquatic Community -** association of interacting assemblages in a given waterbody, the biotic component of an ecosystem (see also aquatic assemblage).

**Aquatic Life Use -** a beneficial use designation in which the waterbody provides suitable habitat for survival and reproduction of desirable fish, shellfish, and other aquatic organisms.

**Attribute -** a measurable component of a biological system.

**Benthic macroinvertebrates -** see benthos.

**Benthos -** animals without backbones, living in or on the sediments, a size large enough to be seen by the unaided eye, and which can be retained by a U.S. Standard No. 30 sieve (28 openings/inch, 0.595-mm openings). Also referred to as benthic macroinvertebrates, infauna, or macrobenthos.

**Bioavailability -** degree to which chemicals can be taken up by organisms.

**Biodiversity -** Refers to the variety and variability among living organisms and the ecological complexes in which they occur. Diversity can be defined as the number of different items and their relative frequencies. For biological diversity, these items are organized at many levels, ranging from complete ecosystems to the biochemical structures that are the molecular basis of heredity. Thus, the term encompasses different ecosystems, species, and genes.

**Biological Assessments or Bioassessments -** evaluation of the biological condition of a waterbody using biological surveys and other direct measurements of resident biota in surface waters.

**Biological Criteria or Biocriteria -** narrative or numeric expressions that describe the biological condition (structure and function) of aquatic communities inhabiting waters of a designated aquatic life use. Biocriteria are based on the numbers and kinds of organisms present and are regulatory-based biological measurements.

**Biological Integrity -** the ability of an aquatic ecosystem to support and maintain a balanced, adaptive community of organisms having a species composition, diversity, and functional organization comparable to that of natural habitats within a region.

**Biological Monitoring or Biomonitoring -** use of a biological entity as a detector and its response as a measure to determine environmental conditions. Toxicity tests and ambient biological surveys are common biological monitoring methods.

**Biological Survey or Biosurvey -** collecting, processing, and analyzing a representative portion of the resident aquatic community to determine its structural and/or functional characteristics.

**Bioregion -** any geographical region characterized by a distinctive flora and fauna (see also ecoregion).

**Clean Water Act (CWA) -** An act passed by the U.S. Congress to control water pollution (formerly referred to as the Federal Water Pollution Control Act of 1972). Public Law 92-500, as amended. 33 U.S.C. 1251 et seq.

**Clean Water Act Section 303(d) -** annual report to Congress from EPA that identifies those waters for which existing controls are not sufficiently stringent to achieve applicable water quality standards.

**Clean Water Act Section 305(b) -** biennial reporting requires description of the quality of the Nation's surface waters, evaluation of progress made in maintaining and restoring water quality, and description of the extent of remaining problems by using biological data to make aquatic life use support decisions.

**Community -** all the groups of organisms living together in the same area, usually interacting or depending on each other for existence.

**Criteria -** statements of the conditions presumed to support or protect the designated use or uses of a waterbody. Criteria may be narrative or numeric.

**Density-Dependence -** regulation of the size of a population by mechanisms that are themselves controlled by the size of that population (e.g. the availability of resources) and whose effectiveness increases as population size increases.

**Designated Use -** classification specified in water quality standards for each waterbody or segment describing the level of protection from perturbation afforded by the regulatory programs. The designated aquatic life uses established by the state or authorized tribes set forth the goals for restoration and/or baseline conditions for maintenance and prevention from future degradation of the aquatic life in specific waterbodies.

**Diatom -** microscopic algae with cell walls made of silicon and have two separating halves.

**Ecological Integrity -** the condition of an unimpaired ecosystem as measured by combined chemical, physical (including physical habitat), and biological attributes.

**Ecoregions -** a relatively homogeneous ecological area defined by similarity of climate, landform, soil, potential natural vegetation, hydrology, or other ecologically relevant variables (see also bioregions).

**Habitat -** a place where the physical and biological elements of ecosystems provide a suitable environment including the food, cover, and space resources needed for plant and animal livelihood.

**Historical Data -** data sets from previous studies, which can range from handwritten field notes to published journal articles.

**Impact -** change in the chemical, physical (including habitat) or biological quality or condition of a waterbody caused by external sources.

**Impairment -** detrimental effect on the biological integrity of a waterbody caused by an impact that prevents attainment of the designated use.

**Index of Biological Integrity (IBI) -** an integrative expression of site condition across multiple metrics. An index of biological integrity is often composed of at least seven metrics. The plural form is either indices or indexes.

**Macroinvertebrates -** animals without backbones of a size large enough to be seen by the unaided eye and which can be retained by a U.S. Standard No. 30 sieve (28 meshes per inch, 0.595 mm openings).

**Metric -** A calculated term or enumeration representing some aspect of biological assemblage, function, or other measurable aspect and is a characteristic of the biota that changes in some predictable way with increased human influence. A multimetric approach involves combinations of metrics to provide an integrative assessment of the status of aquatic resources.

**Microinvertebrates -** animals without backbones that are not large enough to be seen by the unaided eye; they will not be retained by a U.S. Standard No. 30 sieve (28 meshes per inch, 0.595 mm openings).

**Minimally Impaired -** sites or conditions with slight anthropogenic perturbation relative to the overall region of the study.

**Multimetric -** analysis techniques using several measurable characteristics of a biological assemblage.

**Multivariate Community Analysis -** statistical methods (e.g. ordination or discriminant analysis) for analyzing physical and biological community data using multiple variables.

**Narrative Biological Criteria -** general statements of attainable or attained conditions of biological integrity and water quality for a given designated aquatic life use.

**Non-Point Source Pollution -** pollution that occurs when rainfall, snowmelt, or irrigation runs over land or through the ground, picks up pollutants, and deposits them into rivers, lakes, and coastal waters or introduces them into ground water.

**NPDES -** National Pollutant Discharge Elimination System.

**Numeric Biocriteria -** numerical indices that describe expected attainable community attributes for different designated aquatic life uses.

**Point Source -** origin of a pollutant discharge from a discrete conveyance typically thought of as an effluent from the end of a pipe.

**Population -** aggregate of individuals of a biological species that are geographically isolated from other members of the species and are actually or potentially interbreeding.

**Reference Condition -** set of selected measurements or conditions of unimpaired or minimally impaired waterbodies characteristic of a waterbody type in a region.

**Reference Site -** specific locality on a waterbody which is unimpaired or minimally impaired and is representative of the expected biological integrity of other localities on the same waterbody or nearby waterbodies.

**Regionalization or Ecoregionalization -** procedure for subdividing a geographic area into regions of relative homogeneity in ecological systems or in relationship between organisms and their environment.

**Stressors -** physical and biological factors that adversely affect aquatic organisms.

**Taxa -** a grouping of organisms given a formal taxonomic name such as species, genus, family, etc.

**Total Maximum Daily Loads (TMDLs) -** calculation of the maximum amount of a pollutant that a waterbody can receive and still meet water quality standards and an allocation of that amount to the pollutant's source.

**Use Attainability Analysis (UAA) -** analysis that describes factors limiting designated use of waterbodies.